COMPARISON OF CONVENTIONAL PAPANICOLAOU, MODIFIED PAPANICOLAOU AND GRAM-STAINED VAGINAL SMEARS IN THE SCREENING OF BACTERIAL VAGINOSIS IN WOMEN ATTENDING THIKA LEVEL 5 (COUNTY) HOSPITAL

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Comparison of Conventional Papanicolaou, Modified Papanicolaou and Gram-stained vaginal smears in the screening of bacterial vaginosis in women attending Thika Level 5 (County) Hospital

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature:_____ Date:_____

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This thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

I dedicate this project to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding; to my children, who have been affected in every way by this quest and to my family and friends, who have encouraged me all the way. My love for you all can never be quantified.

Thank you. May God Almighty bless you abundantly.

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LIST OF ABBREVIATIONS AND ACRONYMS

BV	Bacterial vaginosis
DPX	Dextrene Plasticizer Xylene
ERC	Ethics and Research Committee
HIV	Human Immunodeficiency Syndrome
ICF	Informed Consent Form
IUCD	Intrauterine contraceptive device
KNH	Kenyatta National Hospital
Modified Pap	Rapid, Economic, Acetic acid, Papanicolaou (REAP) method
NACOSTI	National Commission for Science Technology and Innovation
NPV	Negative Predictive Value
Pap	Papanicolaou
pc (s)	Piece(s)
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PPV	Positive Predictive Value
ROC	Receiver Operating Characteristic
Spp	Species
STDs	Sexually Transmitted Disease(s)
STIs	Sexually Transmitted Infection(s)
UoN	University of Nairobi

ABSTRACT

Bacterial vaginosis (BV) is one of the vaginal infections affecting women of reproductive age group and is associated with many gynecologic and obstetric complications including increased acquisition of Human Immunodeficiency Virus (HIV) infection. Bacterial vaginosis is asymptomatic in up to 50% of women who remain largely undiagnosed which presents a public health concern because of the potential impact of the untreated infection in these women causing increased morbidity and mortality especially in non-pregnant women. The objective of this descriptive cross-sectional study was to compare Conventional Papanicolaou, Modified Papanicolaou (Rapid Economic Acetic acid Papanicolaou-READ) and Gram-stained vaginal smears in screening of bacterial vaginosis in women to establish if Modified Papanicolaou (READ) was a suitable alternative to Conventional Papanicolaou method in this regard. The study findings demonstrated that Gram stain method Nugent's scoring system which was the diagnostic gold standard in this study detected 42 positive cases of bacterial vaginosis out of 150 participants, representing a prevalence of 28% with Conventional Papanicolaou and Modified Papanicolaou (READ) methods demonstrating 25 (16.7%) and 16 (10.7%) positive cases respectively. Using 95% confidence interval and statistically significant p value of $\leq$ 0.05, the study further showed that Conventional Papanicolaou and Modified Papanicolaou (READ) methods had sensitivity of 47.6% and 26.2%, positive predictive value (PPV) of 80.0% and 68.8%, negative predictive value (NPV) of 82.4% and 76.9%, likelihood ratio of positive result (LR+) of 10.3 and 5.69, likelihood ratio of negative result (LR-) of 0.55 and 0.77 respectively with a similar specificity of 95.4% and overall diagnostic accuracy of 38.9%. In addition, the study findings demonstrated a statistically significant kappa value of 0.692 ($p \leq 0.05$) that showed moderate agreement in the diagnostic efficiency capabilities of Conventional Papanicolaou and Modified Papanicolaou (READ) methods. In conclusion, the study showed that Modified Papanicolaou (READ) method is a suitable alternative to Conventional Papanicolaou method in screening of bacterial vaginosis in vaginal smears. This study recommends addition of Modified Papanicolaou (READ) method in screening of bacterial vaginosis in asymptomatic cases where the gold standard is not available.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Bacterial vaginosis (BV) is among the most common lower genital tract infections affecting women worldwide (Kamga et al., 2019) and is observed in many types of clinics such as primary care units, sexually transmitted diseases (STD) clinics, and abortion clinics (Ling et al., 2013; Alcaide et al., 2016; Zhang et al., 2017). It is a vaginal infection caused by imbalance in the normal vaginal flora (Puran et al., 2014; Harb, 2020), where there is low levels of normally predominant *Lactobacilli species (spp),* which is replaced by *Gardnerella vaginalis, Prevotella spp, Porphyromona spp, Bacteroides spp, Mobiluncus spp* and *genital Mycoplasma spp* (Demba et al., 2005; Redelinghuys et al., 2020).Until recently, BV which was thought to be of little long-term clinical significance, has been implicated in adverse reproductive health outcomes (Joshi et al., 2020) such as increase in the risks of preterm birth, still births, preterm birth, premature rupture of membranes, preterm labour, development of pelvic inflammatory disease, pregnancy loss, gestational bleeding, amniotic fluid infection , postpartum endometritis and post caesarean wound infections (Nugent et al., 1991; Lachiewicz et al., 2015; Kotdawala et al., 2019).

Oral squamous cell carcinoma (OSCC) is a common oral malignancy of the head and neck (Almangush et al., 2020) and is ranked thirteenth in terms of mortality but fourteenth in terms of incidence among other cancers. OSCC alone considered as responsible for more than 90% of oral cancers cases and have the highest rate of mortality globally (Bugshan et al., 2020). OSCC may affect numerous anatomical structures such as the lips, tongue, upper and lower gingiva, retromolar triangle, alveolar mucosa, floor and roof (palate) of the mouth, buccal mucosa, oropharynx and the salivary glands (Force et al., 2019). OSCC usually appears on the lateral border of the tongue 40%, followed by the floor of the mouth 30% and the lower lip (Givony, 2020). OSCC develops due to many etiological factors, but smoking and alcohol remain the most common risk factors especially in the Western world

(Tenore et al., 2020). In South Asian countries, consumption of smokeless tobacco and areca nut products are the main etiological factors associated with OSCC (Siddiqi et al., 2020). Most of the oral and oropharynx OSCC cases occur in elderly male patients, with tonsils and tongue being the most commonly affected sites (Anwar et al., 2020).

BV is a cervico-vaginal infection that has repeatedly been associated with Cervical Intraepithelial Neoplasis (CIN) and Human Papilloma Virus (HPV) due to its characteristic of having disturbed vaginal bacterial ecosystem (Biswal et al., 2014). In a study by Watts et al. (2005), BV was associated with incident and prevalent HPV infection and strong evidence suggests this as indicated in a meta-analysis review by Gillet et al. (2011), where there was a positive association between BV and uterine cervical HPV infection (OR, 1.43; 95% confidence interval, 1.11-1.84). In a study by Lu et al. (2015), the findings showed that BV and HPV infections may be synergistic since BV was common in HPV positive subjects and in turn HPV infection was common in BV positive subjects. Additionally, majority of HPV/BV positive cases presented with CIN and cervical cancer. This is further advanced by Zhang et al. (2017) who reported that BV was associated with an increased risk of high-risk HPV (HR-HPV) ($P < 0.0001$; odds ratio, 3.0 (95% CI, 1.7-5.4). However, in another study by Nam et al. (2009), there was significant correlation between BV and the presence of CIN but no significant association between the presence of BV and HPV infection.

This mounting scientific evidence has added to the need for routine gynaecologic examination to include BV screening (Cancer network, 1995) as supported by a study by Farr et al. (2015) whose findings showed that the incidence of preterm birth was 9.7% ($p<0.001$) with reduced low-birth weight neonates, stillbirths, and late miscarriages in women who participated in an antenatal BV infection screen-and-treat program compared to 22.3% ($p < 0.001$) in the women who did not participate in this program.

1.2 Problem statement

BV is the most common cause of vaginal discharge or malodour. However, up to 50% of women with BV may not report symptoms (Centre for Disease Control and Prevention, 2002) resulting in diagnosis of BV mostly in symptomatic women

despite its demonstrated gynecologic and obstetric complications (Kamga et al., 2019). Furthermore, BV has been associated with increased susceptibility to HIV infection and other STDs, and has been implicated in HIV transmission (Morris et al., 2001). Therefore, this shows that BV is of public health concern because of its potential impact to increase morbidity and mortality from other diseases especially in non-pregnant women.

1.3 Justification

Conventional Papanicolaou and Modified Papanicoloau (REAP) methods are primarily used to stain cervico-vaginal (Pap) smears to screen for cervical cancer but also detects cervico-vaginal infections (non-neoplastic pathologies) due to bacteria, fungi and candida, with Bethesda reporting system of pap smear results providing for a comment on 'shift in vaginal flora, suggestive of BV'. Since many asymptomatic cases of BV may remain otherwise undiagnosed and hence not treated, the widespread use of cervical cancer screening campaigns to detect cervical anomalies may help to detect BV to prevent development of complications in future. Baka et al. (2013) reported that observation of clue cells, suggestive of BV, in 30-50% of Pap smears showing inflammation raises concern and advocates for the use of Pap smears to screen for BV associated cervical infections. In a prospective cohort study done over 20 years in Mombasa, Masese et al. (2015), showed that BV is a co-factor to HIV acquisition among high-risk women and hence preventive measures against BV would help the situation. Roeters et al. (2010) alluded to the fact that detection of cervical infections can be a valuable by-product of screening and this gives strong support to scale up screening of BV in routine gynaecological examination.

Validation of Papanicolaou method in screening of BV has already been reported by many authors (Giacomini et al., 1998; Livengood, 2009; Filho et al., 2010; Hodiwala et al., 2015). However, few studies exist in our local setup validating use of Modified Papanicolaou (REAP) method in screening of BV in vaginal smears. Modified Papanicolou (REAP) method has been shown to be cost effective compared to Conventional Papanicolaou method in resource poor settings due to use of less alcohol and reduced turnaround time in its protocol (Gachie et al., 2011). For this reason, it is therefore necessary to make statistical comparisons between these two tests against an acceptable gold standard in randomly selected subjects from the same

population. Therefore, this study will endeavour to validate Modified Papanicolaou (REAP) method in screening of BV in vaginal smears by comparing its diagnostic efficiency statistics to those of Conventional Papanicolaou method against Gram stain as the gold standard to establish if Modified Papanicolaou (REAP) method is a wholly adequate alternative to Conventional Papanicolaou method in screening of BV in vaginal smears in the absence of the gold standard. This study will provide evidence based information to the body of scientific literature on performance of Modified Papanicolaou (REAP) method in screening for BV in vaginal smears; provide scientific world with pilot site for further research; address some of the health system inadequacies such as cheaper screening methods for BV; inform health care management system of the need to include posterior fornix and lateral vaginal wall specimen sourcing sites into routine gynaecologic examination and eliminate the need for further vaginal sample collection for microbiological tests hence avoiding duplication of tests.

1.4 Research question

Is there a difference in screening of bacterial vaginosis in vaginal smears stained using three different staining methods of Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain?

1.5 Objectives

1.5.1 General Objective

To compare Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram-stained vaginal smears in screening of bacterial vaginosis in women attending Thika Level 5 (County) Hospital.

1.5.2 Specific Objectives

1. To screen bacterial vaginosis in vaginal smears using Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain methods.
2. To determine sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratios and diagnostic accuracy of Conventional Papanicolaou and Modified Papanicolaou (REAP) methods to establish if Modified Papanicolaou (REAP) method is a suitable alternative to Conventional Papanicolaou method in screening of BV in vaginal smears.

1.6 Theoretical and conceptual framework

1.6.1 Theoretical framework

A *screening test* is a medical procedure performed on individuals of a defined asymptomatic population to identify those who may have a particular disease. In most cases, screening tests *do not diagnose the illness* and a positive screening test is usually followed up by a diagnostic test that determines the presence or absence of a disease in symptomatic individuals to establish a definitive diagnosis (*www.dictionary.com/browse/screening-test*).

Wilson and Jungner (1968) described a ten-criterion guide for a good screening program that has been adopted by WHO to date. The medical condition being screened for should have the following characteristics; it should be an important health problem; it should have accepted treatment, agreed policy on whom to treat and facilities for treatment should be available; natural history of the disease from latent or early symptomatic stage to development of full-blown disease should be clearly understood and recognizable; case-finding should be continuous but cost effective; a suitable screening test or examination that is acceptable to the population and facilities for diagnosis for the condition should be available.

Validation of screening test is important at establishing the suitability of the test in detecting subjects with or without the disease or condition of interest. Validation of screening test is often done by comparison of the screening test results against the true test status of the subjects obtained using a generally accepted gold standard which under reasonable conditions, is considered a definitive diagnostic standard (Greenhalgh, 1997). Evaluation of the performance of a diagnostic/screening test involves objective measures called diagnostic efficiency statistics, which assess the discriminative and predictive abilities of the test. They include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratio of a positive test (LR+) and likelihood ratio of a negative test (LR-) (Greenhalgh, 1997; Akobeng, 2006; Šimundić, 2009; Mandrekar, 2010).

1.6.2 Conceptual framework

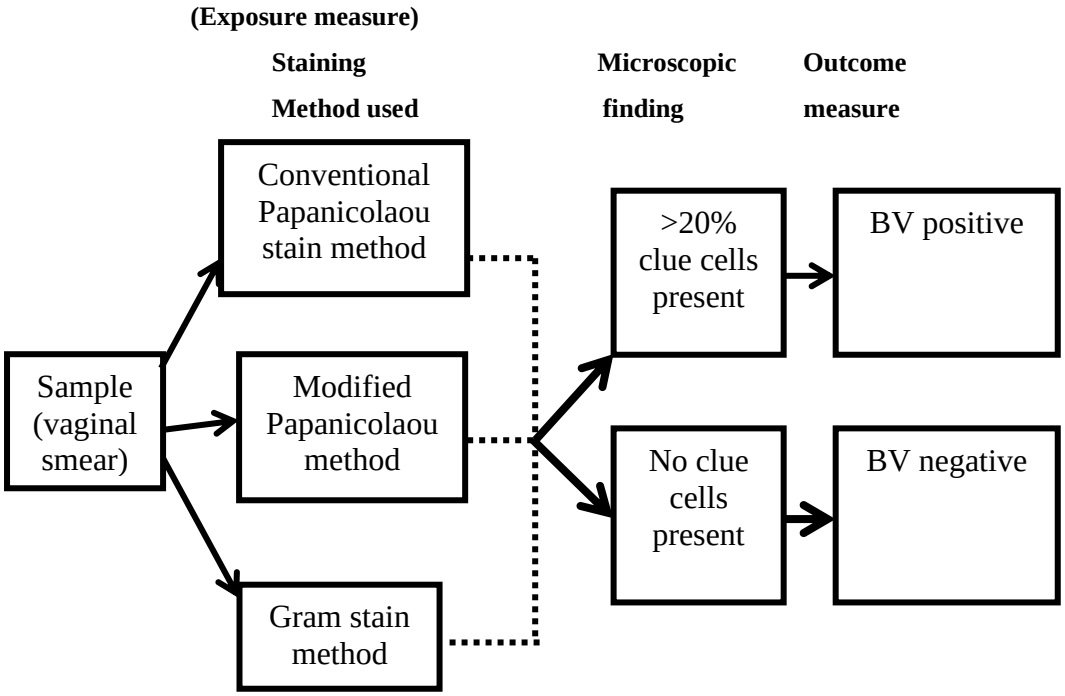


Figure 1.1: Conceptual framework to show BV screening plan for the sample

The sample (vaginal smear) will be subjected to three staining methods, Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain for subsequent microscopic examination for clue cells, whereby presence of >20% clue cells will indicate positive BV test and absence of clue cells will indicate negative BV test.

CHAPTER TWO
LITERATURE REVIEW

2.1 History of BV

Bacterial vaginosis (BV) was first reported in 1955 by Gardner and Dukes (Chaim et al., 1997; Turovskiy et al., 2011; Bautista et al., 2016). They also named the causative agent as *Gardnerella vaginalis*. It is currently the most common cause of infectious vaginitis in women with reproductive tract disease (Gillet et al., 2011; Aminzadeh et al., 2013; Narayankhedkar et al., 2015). It is caused by a shift in normal vaginal flora resulting in increased vaginal pH, grey, homogenous, offensive vaginal discharge and 'clue cells' (represent epithelial cells with excess bacteria attached on their surfaces). Most of the patients are asymptomatic but if untreated, develop a copious malodorous discharge (Aviles et al., 1999; Onderdonk et al., 2016).

2.2 Prevalence of BV

Due to dynamic nature of populations in different geographical regions and countries, there is no agreeable global prevalence of BV even though they are apparent between and within different populations. In previous studies, the prevalence of BV has been reported to vary between and within populations with a wide range of 10%-50% in pregnant women (Redelinghuys et al., 2015). Estimated prevalence of BV ranges from 20% to 50% in African populations (Sobel, 2000), with higher levels being documented in female sex workers (Fonck et al., 2001; Riedner et al., 2003). Women in sub-Saharan Africa have 55% prevalence rate (Woodman, 2016) with a study in Ghana reporting a prevalence rate of 28% (Aubyn et al., 2013).

Its frequency differs from country to country and across different populations within countries but is reported to be; 24-37% (Finch et al., 2010) and 8-75% (Adane et al., 2017) in women attending sexually transmitted infection (STI) clinics), 15-20% in pregnant women (Money, 2005), and 51.5 % in women attending gynaecology clinics (Baruah et al., 2014).

In Kenya, BV prevalence rate has been documented as 44% (Bukusi et al., 2006) but various studies have proposed varying rates within Kenya. A study in Thika district hospital reported a prevalence of 26.0% (95% CI: 34.2-48.6) (Nzomo, 2011) and

43.1%, (95% CI 36.2 - 50.1) (Nzomo et al., 2013); while another study among pregnant women in a rural county hospital in Kilifi reported a prevalence rate of 19.3% (95% CI: 14.1-25.4) (Masha et al., 2017); while a cross-sectional study in Western Kenya reported a prevalence of 48.3% (Menon et al., 2016). In a respondent driven sampling (RDS) study among female sex workers (FSW) in Nairobi, Kenya, Musyoki et al. (2015) reported a BV prevalence of 15.1%. This is slightly different from a study on FSW in Kisumu, Western Kenya by Vandenhoudt et al. (2013) that reported a 27.0% BV prevalence. A longitudinal study conducted in Kenya, Rwanda and South-Africa reported BV prevalence of 38% in women at the screening visit (Jespers et al., 2014). Further, in a clinical trial involving a cohort of HIV-1 serodiscordant heterosexual couples from Southern and East Africa, an association of BV and HIV transmission was reported through the finding of an incidence of 2.91/100 persons of HIV-1 infection in men whose female partners, already infected with HIV-1, had BV, in comparison to an incidence of 0.76/100 persons HIV-1 infection in men whose female partners, already infected with HIV-1, had normal vaginal flora (Cohen et al., 2012). Similarly, a perinatal cohort study in Nairobi involving HIV-1 infected pregnant women revealed 37% BV prevalence (Marx et al., 2010).

2.3 Vaginal flora

Normal bacterial flora of the vagina including illustration of the bacteria was first described in a publication by Doderlein in 1892 (Linhares et al., 2010; Martin, 2012; Rampersaud et al., 2012; Bautista et al., 2016). These bacteria were shown to be facultative anaerobic Gram-positive bacteria and form part of a group of bacteria called *Lactobacilli* (Srinivasan et al., 2008; Bautista et al., 2016; Onderdonk et al., 2016). In 1898, Kronig reported a motile rod that he believed was normal vaginal flora. This motile rod was later on described as a bacterium called *Mobiluncus* species (Martin, 2012). In 1921, Shroder proposed a three-grade system for vaginal microflora. In grade I, *Lactobacilli* dominate the normal/healthy vaginal flora; in grade II, there is partial replacement of *Lactobacilli spp*. with other bacteria (usually anaerobes such as *Mobiluncus*, *Bacteroides*, *Prevotella*, *Peptostreptococci*, *Eubacterius* and *Mycoplasma hominis*) and in grade III, *Lactobacillus spp.* is absent and completely replaced by other bacteria (Donders et al., 1996; Bautista et al.,

2016). Presence of these anaerobic rods results to vaginal discharge (Srinivasan et al., 2008; Martin, 2012; Abdelaziz et al., 2014). This abnormal vaginal discharge was referred to as a syndrome called non-specific vaginitis because a specific causative agent of the condition had not yet been identified (Kumar et al., 2011; Machado et al., 2015). However, in 1955, *Haemophilus vaginalis* (*Gardnerella vaginalis),* was isolated from women with non-specific vaginitis by Gardner and Dukes (Salmon et al., 1991; Jarosik, 1998; Jayaprakash et al., 2012) and called this syndrome *Haemophilus vaginalis* vaginitis (Hickey et al., 2014; Schwebke et al., 2014). However, other studies showed that there was presence of low concentrations of *G. vaginalis* in clinically healthy women who did not have *Haemophilus vaginalis* vaginitis (Totten et al., 1982; Hickey et al., 2014; Schwebke et al., 2014; Janulaitiene et al., 2017). Due to this, the syndrome was named bacterial vaginosis in 1983 (Bautista et al., 2016) and it referred to a condition where there is replacement of *lactobacilli* of the vagina by characteristic groups of bacteria that result in changes in the properties of vaginal fluid (Tamrakar et al., 2007; Cribby et al., 2008; Petricevic et al., 2014). The major bacteria involved in bacterial vaginosis are *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma urealyticum* and *Mobiluncus* species (Malaguti et al., 2015).

2.4 Pathogenesis of BV

Bacterial vaginosis (BV) is due to a change in normal vaginal flora and is characterized by reduction in number of normally dominant *lactobacilli* accompanied by increase in number of other bacteria especially anaerobic Gram-negative rods (Jogi et al., 2015). *Lactobacilli* produce hydrogen peroxide that maintains vaginal pH and prevents overgrowth of anaerobic bacteria present in vaginal flora (Vitali et al., 2007; Haya et al., 2014; Tachedjian et al., 2017). Loss of *lactobacilli* results in increase in vaginal pH and increased overgrowth of vaginal anaerobic bacteria that produce huge amounts of proteolytic carboxylase enzymes (Truter et al., 2013; Zetian et al., 2015). These enzymes break down vaginal peptides into various amines (Nelson et al., 2015) which are similar to amines produced by bacteria that spoil fish for example trimetylamine (TMA), which are dissolved in the vaginal discharge as an acid when the pH is low (Yeoman et al., 2013). These amines are volatile, produce an odour similar to the smell of spoiled fish and lead to increased vaginal

discharge and exfoliation of squamous cells resulting in clinical features of BV (Brotman, 2011; Biswal et al., 2014; Dasari et al., 2014). In addition, there is attachment of *G. vaginalis* to form a biofilm over the exfoliating epithelial cells (Gilbert et al., 2013; Machado et al., 2015) and increasing evidence suggests that *G. vaginalis* is the key causative agent in the pathogenesis of BV (Patterson et al., 2010; Muzny et al., 2016; Janulaitiene et al., 2017; Younus et al., 2017).

2.5 Signs and symptoms of BV

Patients with BV may present with a variety of symptoms or none at all (Dingens et al., 2016; Hilbert et al., 2016; Akinajo et al., 2017). More than 50% of all women with BV may be asymptomatic (Turovskiy et al., 2011; Brooks-Smith-Lowe et al., 2013; Hoffman et al., 2014; Tamunomie et al., 2015). In about 50-70% of all patients, the major symptom is an unpleasant, fishy or musty odour (Nwadioha et al., 2011) which is made worse following sexual intercourse (Fethers et al., 2012; Mascarenhas et al., 2012; Ahmed et al., 2014) or during menstruation because of increase in vaginal pH (Chen et al., 1979; Truter et al., 2013). In addition, increased vaginal discharge is common (Rafiq et al., 2015). This discharge is usually thin (Gilbert et al., 2013; MengiStie et al., 2013; Malaguti et al., 2015), gray (Kumar et al., 2011) or white/milky (Kumar et al., 2011; MengiStie et al., 2013; Machado et al., 2015) and homogeneous (Kumar et al., 2011; MengiStie et al., 2013; Machado et al., 2015; Malaguti et al., 2015) and it tends to adhere to the vaginal wall (Korenek et al., 2003). In some cases, vaginal itching and irritation may be present (Onderdonk et al., 2016). Odour and discharge are two of the four diagnostic criteria used by Amsel in his clinical composite criteria for the diagnosis of BV (Mahajan et al., 2017). BV is also associated with more serious disease (Klebanoff et al., 2010; Machado et al., 2016) and may affect the cervix causing acute cervicitis (Marrazzo, 2006; Alcendor, 2016; Klein et al., 2019) characterized by endocervical mucopurulent discharge or easily induced cervical bleeding (Marazzo et al., 2006; Tarney et al., 2014; Gorgos et al., 2015).

2.6 Risk factors of BV

BV is caused by factors that lower vaginal pH which interferes with the natural ecology of the vagina (Biswal et al., 2014). These include factors such as insertion of traditional herbs into the vagina (Allen et al., 2010; Hilber et al., 2010; McCarthy et

al., 2015), douching (Bleicher et al., 2015; Tamunomie et al., 2015; Alcaide et al., 2015), absence of *Lactobacillus* (Falagas et al., 2007; Cribby et al., 2008; O'Hanlon et al., 2011; Alcendor, 2016), low socioeconomic status (Kalinka et al., 2002; Smart et al., 2004; Allsworth et al., 2011; Mengistie et al., 2014), use of intrauterine contraceptive devices (IUCD) (Madden et al., 2012; Shobeiri et al., 2014; Seth et al., 2017), multiple sexual partners (Alesna et al., 1996), increased frequency of sex (Verstraelen et al., 2010; Mascarenhas et al., 2012), African ethnicity (Madden et al., 2012; Akinajo et al., 2017), new sexual partner (Fethers et al., 2008) and level of education (Van de Wijgert et al., 2000; Holzman et al., 2001). Sexual activity is considered a risk factor and it is believed that BV does not occur in women who have never had vaginal intercourse (Fethers et al., 2009). A systematic review and meta-analysis study concluded that since BV is directly proportional to the number of sexual contacts with new and multiple sexual partners (Mitchell, 2004; Forcey et al., 2015), both male and female, it can be reduced by avoiding unprotected sexual encounters (Fethers et al., 2008; Gallo et al., 2011).

2.7 Complications of BV

BV, though in most cases it remains asymptomatic in about 50% of the women (Sweet, 2000; Mitchell, 2004; Filho et al., 2010; Begum et al., 2011; Alice et al., 2012; Hilbert et al., 2016), results in local discomfort and complications in both pregnant and non-pregnant women (Truter et al., 2013; Africa et al., 2014). Gynecologic complications in non-pregnant women include infertility (Gallo et al., 2011; Tamunomie et al., 2015), endometritis, pelvic inflammatory disease (Begum et al., 2011; Taylor et al., 2013; Ibrahim et al., 2017), post-abortal sepsis, post-hysterectomy infection (Lachiewicz et al., 2015), increased risk of HIV (Morris et al., 2001; Atashili et al., 2008; Mascarenhas et al., 2012; Alcaide et al., 2016; Alcendor , 2016) and other STIs acquisition (Gilbert et al., 2013; Imade et al., 2014; Francis et al., 2016). Obstetric complications linked to BV include pregnancy loss (Swidsinski et al., 2013), still births, preterm birth (Gilbert et al., 2013; Isik et al., 2016), preterm labour (Ralph et al., 1999), premature rupture of membranes (Kirmizi et al., 2013; Nakubulwa et al., 2015), amniotic fluid infection (Lata et al., 2010; Mendz et al.,2013), postpartum endometritis (Gupta et al., 2013; Isik et al., 2016) and post caesarean wound infections.

2.8 Diagnosis of BV

There are two main categories of diagnostic tests used to diagnose BV; these are clinical based and laboratory based diagnostic tests (Money, 2005; Kumar et al., 2011). Amsel's clinical criteria and laboratory-based Nugent gram staining evaluation methods are considered as the two main gold standards for diagnosing BV (Mahajan et al., 2017).

2.8.1 Clinical based diagnosis

Amsel's criteria, developed by Amsel et al. (1983) is the most widely accepted clinical criteria and which is still in use today for both pregnant and non-pregnant women. These require that a positive clinical diagnosis of BV is indicated by presence of three out of four of the clinical signs (Khan et al., 2007; Frobenius et al., 2015; Malaguti et al., 2015; Mohammadzadeh et al., 2015).

These signs are: -

a) Vaginal discharge

The discharge should be thin, homogenous, adherent, milky, and evenly coats the vaginal walls (Prasad *et al.*, 2016). A normal discharge is floccular (Money, 2005).

b) Vaginal pH

Vaginal fluid pH greater than 4.5. The pH is measured using pH indicator paper (Hemalatha et al., 2013; Hoffman et al., 2017). This can be determined directly by using pH sticks placed on the vaginal wall (Frobenius et al., 2015; Hoffman et al., 2017) or a vaginal swab placed on pH paper to touch the range covering pH 3.5-5.2 (Hemalatha et al., 2013) or pH 3.5-5.5 (Hoffman et al., 2017).

c) Fishy odour

This can be done in two ways: the first method is trimethylamine sniff test/whiff test which was proposed by Gardner and Duke (1955) where one drop of 10% potassium hydroxide (KOH) is added to the vaginal discharge on the speculum and the odour is detected by smelling directly from the speculum. In the second method proposed by Amsel et al. (1983), there is addition of 10% potassium hydroxide to the sniff test and is performed by placing a drop of vaginal discharge onto a microscope slide, add one drop of 10-20% potassium hydroxide, mix and smell the preparation (Money, 2005).

d) Presence of clue cells

A drop of vaginal discharge is placed on a microscope slide and a drop of saline added. The preparation is covered with a cover-glass and examined at 400x magnification using a light microscope. Clue cells are identified as vaginal epithelial cells whose peripheral borders are difficult to observe because they are coated with many coccobacilli bacteria. A positive diagnosis of BV is indicated by presence of clue cells on >20% of the total vaginal epithelial cell population on microscopic cellular examination of the wet mount of vaginal fluid (Discacciati et al., 2006; Sachdeva, 2006; Frobenius et al., 2015).

Amsel's criteria is simple, easy to perform and does not require expensive equipment and provides an immediate answer; however, it is only applicable in symptomatic women and even in this case, the diagnosis of BV has a subjective endpoint and its sensitivity requires observation of at least presence of three out of the four signs (Gomaa et al., 2017). In addition, the signs are not consistent or reproducible in all the cases (Nugent et al., 1991).

2.8.2 Laboratory based diagnosis

2.8.2.1 Gram staining

Gram stain is a microbiological method used to classify bacteria as either Gram positive or Gram negative. In diagnosis of BV, Gram stain method classifies bacteria as either *Gardnerella* morphotypes or *Lactobacillus* morphotypes which are short bacteria (gram negative or gram variable) and gram-positive rods respectively (Chawla et al., 2013; Machado et al., 2015). It is also known as microbiological diagnosis and involves classification of bacteria morphotypes associated with BV using Nugent's scoring system, which has been widely accepted as the gold standard in the diagnosis of BV in both negative and positive cases, during microscopic examination of Gram-stained vaginal smears (Nugent et al., 1991).

In the procedure by Nugent et al. (1991), the vaginal swab is obtained from the lateral vaginal wall and rolled onto a glass slide to make a thin smear. The smear is heat fixed, gram stained and counterstained with safranin. Each Gram-stained smear will be evaluated for the following morphotypes under oil immersion (x1000 magnification): large gram-positive rods (*Lactobacillus* morphotypes), small gram-variable rods (*G. vaginalis* morphotypes), small gram-negative rods (*Bacteroides*

spp. morphotypes), curved gram-variable rods (*Mobiluncus* spp. morphotypes), and gram-positive cocci. Large gram-negative rods and gram-negative cocci are also noted (Mahajan et al., 2017). Classification of each morphotype is then done using Spiegel (Hodiwala *et al.*, 2015), Nugent (Nugent et al., 1991*;* Chawla et al., 2013), Hay/Ison (Ison et al., 2002; Antonucci et al., 2017) or Ison/Hay classification systems (Ison et al., 2002).

a) Spiegel classification system

In this system, *Lactobacillus* morphotypes and *Gardnerella* morphotyes detected are scored as 1+, 2+, 3+ and 4+ based on the amount of the bacteria seen in the Gram-stained smear. Positive diagnosis of BV is indicated by presence of 1+ to 2+ *Lactobacillus* morphotypes and predominance of *Gardnerella* morphotypes over *Lactobacillus* morphotypes. Negative diagnosis of BV is indicated by presence of only *Lactobacillus* morphotypes in the smear (Nugent et al., 1991).

b) Nugent classification system

It is considered as the gold standard in laboratory-based diagnosis of BV using Gram-stained smears examined at 1000x magnification using oil immersion objective. It is based on detection and rating of different bacterial morphotypes in a point estimation system of 0 to 4 points. Presence of more than 30 *Lactobacilli* morphotypes per vision field earns 0 points and its absence earns 4 points. Presence of more than 30 small bacteria per vision field earns 4 points and its absence earns 0 points. In addition, presence and number of curved rods per vision field earns additional 1 or 2 points. The above points are then added together to get total score. A total score of 0 to 3 is classified as normal, 4 to 6 is intermediate and 7 to 10 is positive for BV (Nugent et al., 1991).

Gram stain Nugent's criteria is time consuming, subjective since it is based on the skill and experience of the person reading the smears, requires extensive training of personnel, faces challenges of interpreting intermediate smears (Chawla et al., 2013) and does not provide specific bacteria species assessment in the vaginal microbiota (Shipitsyna et al., 2013). As a result, other scoring systems have been proposed such as Spiegel's, Hay/Ison, Ison/Hay and Schmidt's (Muthusamy et al., 2016; Onderdonk, 2016) with the adoption in many cases of Hay/Ison scoring system (Antonucci et al., 2017).

Table 2.1: Nugent scoring system

Score	*Lactobacillus* morphotype/vision field	*Gardnerella* morphotype/vision field	Curved bacteria morphotype/vision field
0	*>30*	0	0
1	5-30	<1	1-5
2	1-4	1-4	>5
3	<1	5-30	
4	0	>30	

Scores

0-3 Normal flora

4-6 Intermediate flora

7-10 BV

Source: MAMC Journal of Medical Sciences

a) Hay/Ison classification system

It is used for both Gram stained and Pap-stained vaginal smears. This classification system divides vaginal flora into normal, intermediate and BV categories. It is based on comparison of the amounts of bacteria present in the smear. It can be used to evaluate slides stained with Gram or Pap staining methods and also unstained smears (Ison et al., 2002).

Table 2.2: Hay/Ison classification

	***Lactobacilli* morphotypes**	***Gardnerella* morphotypes**
Normal (group 1)	Many	Few
Intermediate (group 2)	Equal amount	Equal amount
BV (group 3)	Few	Many

Source: BioMed Research International (2013)

b) Ison/Hay classification system

It can be used to evaluate slides stained by Gram or Pap staining methods and also unstained smears. It has five categories: Group 0, normal (group 1), intermediate (group 2), BV (group 3) and Group 4 (dominance of *Streptococcus* morphotype) (Ison et al., 2002).

Table 2.3: Ison/Hay classification

	***Lactobacilli* morphotypes**	***Gardnerella* morphotypes**
Group 0	None	None
Normal (group 1)	Many	Few
Intermediate (group 2)	Equal amount	Equal amount
BV (group 3)	Few	Many
Group 4 (dominance of *Streptococcus* morphotype)	None	None

Source: Sexually transmitted Infections (2004)

2.8.2.2 Papanicolaou staining

Papanicolaou test (abbreviated as Pap test, Pap smear, cervical smear or smear test) is a cytology-based screening test, discovered by George Nicholas Papanicolaou in 1941 (Tan et al., 2015; Raju, 2016; Ciardullo, 2017). It is considered a gold standard in cervical cytology and its primary purpose is microscopic examination for normal and abnormal cells in the cervix and vagina in the early detection of cervical cancer and precancerous lesions (Mehmetoglu et al., 2010; Rositch et al., 2012; Hodiwala et al., 2015). Conventional Papanicolaou protocol has undergone various modifications to make it cost effective in terms of reducing the turnaround time and quantities of

alcohol utilized in the protocol (RoyBiswas et al., 2008; Gachie et al., 2011). The staining quality and final interpretation of Modified Papanicolaou protocols is not compromised hence its adoption as a suitable alternative to Conventional Papanicolaou in screening for cervical cancer in resource limited setups (Akinremi et al., 2005; Dighe et al., 2006; RoyBiswas et al., 2008; Gachie et al., 2011; Ashok et al., 2015; Vani et al., 2017); but has poor stain preservation for research purposes (Izhar et al., 2014). One of this modification is the Modified Papanicolaou protocol known as Rapid, Economic, Acetic acid, Papanicolaou (REAP) method (RoyBiswas et al., 2008) that has shown no compromise on staining quality and diagnostic standards and has therefore been successfully utilized in screening for cervical cancer in Pap smears (Gachie et al., 2011).

Papanicolaou-stained smear technique has also been widely used in screening of BV with Bethesda system guidelines stipulating for a remark such as 'shift in vaginal flora suggestive of BV' (Bombase et al., 2014) if there is presence of the following parameters: filmy background of small coccobacilli, coccobacilli along the cell margins of individual squamous epithelial cells (clue cells) and conspicuous absence of *Lactobacilli* (Hodiwala et al., 2015). Several studies report presence of clue cells as suggestive of BV (Michael, 1999; Simões-Barbosa et al., 2002; Vardar et al., 2002; Discacciati et al., 2006; Filho et al., 2010; Gillet et al., 2011; Baka et al., 2013; Truter et al., 2013; Puran et al., 2014; Sabu et al., 2017) with a threshold of >20% clue cells (Discacciati et al., 2006; Sachdeva, 2006) while others consider presence of coccobacilli only (Prey, 1999). Recent studies have shown that Papanicolaou stained smears can be used to screen for BV (Simoes-Barbosa et al., 2002, Vardar et al., 2002; Discacciati et al., 2006).

Even though Pap smear has moderate sensitivity but high specificity compared to the microbiological test, it has diagnostic value when it is positive (Tokyol et al., 2004) and if it yields a mean specificity of 95% when compared to gold standard (Filho et al., 2010) especially for the screening of asymptomatic BV (Livengood, 2009; Filho et al., 2010). Several authors support the use of Pap-stained smear techniques for BV diagnosis as a wholly adequate alternative to Gram-stained smears (Giacomini et al., 1998; Hodiwala et al., 2015). However, few validation studies comparing scoring of

Papanicolaou-stain procedure against the Amsel's and Nugent's gold standards have not yet been conducted (Vardar et al., 2002).

2.8.2.3 Culture

Culture of *Gardnerella vaginalis* is another sensitive method for diagnosis of BV (Gergova et al., 2013). However, this method has low specificity because the bacterium has been isolated in up to 50% to 60% of healthy women without clinical signs of BV (Sha et al., 2005). Additionally, it is unreliable as a true indicator of vaginal microbial flora in BV since *G. vaginalis* is not the only microbe associated with BV infection (Tokyol et al., 2004). To add to this, many other bacteria associated with BV are obligate anaerobes whose isolation using conventional culture methods is difficult (Ravel et al., 2011). Therefore, this method leads to over-diagnosis, making it unsuitable when planning treatment or testing after-treatment status. In addition, since BV is due to changes in vaginal flora, vaginal culture has no role in its diagnosis (Balashov et al., 2014).

2.8.2.4 Biochemical test/ rapid tests/ Point of care testing devices (POCT-devices)

It is based on the detection and measurement of microbial enzyme which is produced by anaerobic bacteria, anaerobic gram-negative bacterial rods such as *Bacteroides, Gardnerella,* and *Prevotella* species and viruses, mycoplasma, fungi, and protozoa sialidase (Wiggins et al., 2001). Based on Amsel's clinical criteria, pH and sniff tests, several commercial test kits have been developed and they are marketed as point of care (POC) testing devices (Tucker, 2013) that mostly detect metabolic by-products (bacterial amines) and bacterial sialidase produced by BV associated bacteria like *Prevotella* and *Bacteroides spp*. responsible for BV (Bradshaw et al., 2005; Madhivanan et al., 2014).

POCT-devices detect metabolic by-products sialidase and proline aminopeptidase, produced by bacteria responsible for BV in vaginal secretions. However, detection of these by-products is more useful where understanding of pathogenesis is more important than its clinical diagnosis (Huppert et al., 2012; Tucker et al., 2013).

Table 2.4: Commercial test kits based on Amsel's clinical criteria

Amsel's criteria	Test kit	Tested substance
pH	Careplan Vaginal pH	pH
	Vi-SENSE	pH
	pH Glove	pH
Whiff/sniff test	FemExam	pH + TMA
	QuickVue Advance	pH + Amintest
	Electronic Nose	TMA
	Pipactivity test card	Aminopeptidase
	BV Blue	Sialidase activity test

2.8.2.5 Molecular diagnosis

Molecular methods have also been adopted in the diagnosis of BV to show the polymicrobial causative nature of BV. However, these methods are not rapid, require very expensive equipment and highly trained personnel for practical routine use hence mostly applicable in research (Dumonceaux et al., 2009). They include: -

a) Polymerase chain reaction (PCR)

Polymerase Chain Reaction (PCR) is used to differentiate BV due to bacteria from other vaginal disorders since they are based upon molecular quantification of vaginal microbiota in BV such as *Gardnerella vaginalis*, *Atopobium vaginae* and other BV associated bacteria (BVAB) (Menard et al., 2008, Cartwright et al., 2012). Targeted-PCR assays and broad range-PCR assays have been applied in this quest with targeted-PCR assays like taxon-directed bacterial 16S rDNA PCR aimed at detection of one or more fastidious BVAB species (Fredricks et al., 2007). However, the broad range PCR-assays is more sensitive at detecting common vaginal microbiota than less abundant microbial types. They include broad range bacterial 16S rDNA PCR, multiplex-PCR (M-PCR) and quantitative real-time PCR (qPCR) which quantify various bacteria morphotypes like *Gardnerella vaginalis* from vaginal fluid swabs in both pregnant and non-pregnant women (Hilbert et al., 2016).

b) Fluorescence in situ hybridization (FISH)

Fluorescence in situ hybridization (FISH) of desquamated vaginal epithelial cells in urine sediment has been used to show presence of biofilm of *Gardnerella*

morphotype (Swidsinski et al., 2010) in diagnosis of BV. The use of a more sensitive Peptide Nucleic Acid (PNA) probes in FISH – (PNA-FISH) has greatly improved the specificity and turnaround time of this technique (Machado et al., 2015).

However, these methods are labour intensive and use sophisticated equipment that requires highly trained personnel. It is therefore not suitable for routine use especially in developing countries but for research purposes (Dumonceaux et al., 2009).

c) Deoxyribonucleic acid (DNA) probes

DNA probes have also been used to detect and identify *Gardnerella vaginalis* nucleic acid in vaginal fluid from symptomatic patients (Cartwright et al., 2013).

2.8.2.6 Wet microscopy

Diagnosis of BV has been done using rehydrated dried wet mounts by detecting bacterial morphotypes and clue cells. Rehydrated dried wet mounts refer to air dried vaginal smear on a slide which is later rehydrated and evaluated using Nugent or Hay/Ison classification systems. They provide comparable image to a wet smear but motile organisms are not visible (Platz-Christensen et al., 1995) but requires phase contrast microscope to contrast small bacteria, *Lactobacilli* and clue cells (Donders et al., 2009).

2.8.2.7 Other methods

Gas-liquid chromatography was used by Spiegel et al. (1980 in vaginal washings to show that women with BV have abnormal vaginal acids. In this method, large amounts of vaginal washings are required for analysis. Due to this, Chen et al. 1982 introduced thin-layer chromatography which uses only two millilitres of sterile water mixed with the vaginal secretion to enable determination of the amine's putrescine and cadaverine (Chen et al., 1982).

a) Gas liquid chromatography (GLC)

Spiegel et al. (1980) used GLC on vaginal washings to detect abnormal non-volatile fatty acids with BV infected women showing increased succinate to lactate (Yeoman et al., 2013) chromatographic peak ratio ≥ 0.4 (Honest et al., 2004; Kafi et al., 2012). GLC method was limited by its requirement for large quantities of vaginal washing.

b) Thin layer chromatography (TLC)

Chen et al. (1982) introduced TLC that utilized only 2mls of sterile water mixed with vaginal secretion to detect the amines, diamines, putrescine and cadaverine, responsible for the fishy odour in the potassium hydroxide 'whiff 'test (Nelson et al., 2015).

2.9 Differential diagnoses of BV

In the absence of microscopy, diagnosis of BV is unlikely if there is absence of fishy odor. This is because BV is suspected if the vaginal pH is greater than 4.5 but this pH also indicates other infections like atrophic vaginitis, desquamative inflammatory vaginitis and trichomoniasis. However, BV can be differentiated from these three conditions because of the following facts: women with BV do not have dyspareunia or signs of inflammation; BV does not have increase in number of parabasal cells; microscopy of BV does not show large numbers of polymorphonuclear leucocytes and BV does not show trichomonads (Klauss-Silva et al., 2014).

2.10 Treatment of BV

Treatment of BV is difficult because clinical cure is not universally successful (Dickey et al., 2009). In one-third of non-pregnant and one-half of pregnant women, BV resolves spontaneously while in other cases drugs are used to relieve the symptoms. The drugs include Metronidazole, clindamycin, tinidazole and Secnidazole which are administered orally or intravaginally. Symptomatic relapse should be treated initially with a seven-day course of oral or vaginal metronidazole or clindamycin (Paladine et al., 2018).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Research design

This was a descriptive cross-sectional study of bacterial vaginosis with a comparative evaluation of three methods in screening of BV in vaginal smears in women attending family planning and ante-natal clinics in Thika Level 5 (County) Hospital. This design was selected since the primary goal of the study was to assess the sample of a predefined population at one specific point in time to detect presence/absence of BV. One hundred and fifty (n=150) consenting female participants who met the inclusion criteria were randomly selected and recruited into the study between November 2016 and May 2017. The participants were informed about the study, the procedures to be undertaken, harmful and beneficial effects of the study, privacy and confidentiality measures. Only those women who accepted to take part in the study were required to respond to an interviewer-administered questionnaire upon signing and giving out a written informed consent form. Those participants who were not able to sign were allowed to thumb-print as a sign of consent.

3.2 Study area

The study was conducted at Thika Level 5 (County) Hospital. It is a government hospital located in Kenya, former Central province, Kiambu County, Thika West district, Juja constituency, Thika municipality division, Thika town location, Biashara sub-location. Thika town is about 50 km from Nairobi, the capital city of Kenya. The hospital is headed by the Medical Superintendant and is operational with a bed capacity of about 300 and 24 cots. It offers a wide range of services including laboratory, casualty, family planning, pre- and post-natal services, antiretroviral therapy, curative in-patient services, immunization and HIV counselling and testing. Thika district is one of the seven districts in Central province. The district covers an area of 1,960.2sq Km^2. It borders city of Nairobi to the South, Maragua district to the north, Machakos district to the east and Kiambu district to the west. The district is divided into six divisions; Gatundu South, Ruiru, Thika municipality, Gatanga, Kamwangi (Gatundu North) and Kakuzi. Ruiru is the largest division followed by Kakuzi and Kamwangi while the smallest division is Gatundu South. In addition,

Thika district has four constituencies; Juja, Gatanga, Gatundu North and Gatundu South (National Coordination Agency for Population and Development, 2005).

3.3 Study Population

In 2002, the population of Thika district was estimated to be 701,664 with a growth rate of 2.8% per annum with number of males and females at 351,511 and 350,153 respectively, representing a female/male sex ratio of 1:1. The population of females of reproductive age group of 15-49 years rose from 181,383 in 2002 to a projected figure of 214,180 by the year 2008 (National Coordination Agency for Population and Development, 2005). The population is both urban and rural and highly cosmopolitan. The study targeted all women aged 18-45 years visiting the family planning and antenatal clinics at Thika Level 5 (County) Hospital during the study period. Only those women who met the inclusion criteria and agreed to participate in the study after giving their informed consent were recruited into the study.

3.3.1 Inclusion criteria

i. Females aged 18-45 years (child bearing age), sexually active with malodorous vaginal discharge
ii. No vaginal bleeding at the time
iii. Participants who give consent

3.3.2 Exclusion criteria

i. Used any antibiotics in the last two weeks.
ii. Used vaginal creams in the last two weeks.
iii. Had sexual intercourse within the last 8 hrs
iv. Active vaginal bleeding

3.4 Variables

i. Independent variable- test result, which is either positive or negative
ii. Dependent variable-test result obtained after screening for bacterial vaginosis in vaginal smear

3.5 Sampling procedure

3.5.1 Sampling technique

Simple random sampling was used to select eligible participants after fulfilling the inclusion criteria highlighted above. The sampling was done using Stat Trek's Random Number Generator tool which is found under the Stat Tools tab, which

appears in the header of Stat Trek web page (stattrek.com). Sampling was done without replacement i.e. if a number was selected; it was put aside so that it could be selected only one time to avoid it being selected more than once. This ensured that each individual had equal opportunity of being included in the study.

3.5.2 Sample size determination

The appropriate sample size for the study was calculated using Andrew Fisher's method (1994) for a population above 10000, based on 95% confidence interval. From literature review, there was no documented data on the prevalence and incidence of BV in Kenya. Therefore, the best guess estimate of the prevalence of BV in Kenya was 10% based on an article by Georgijevic et al. (2000) which states that "BV is the most prevalent form of vaginal disturbances in reproductive age women. The average incidence of BV varies: 10-35% in patients visiting gynaecological wards, 10-30% in patients visiting obstetric wards and 20-60% in patients visiting services of sexually transmitted diseases."

Formula, $n=\frac{z^2pq}{d^2}$

Where

n= minimum number required

z= level of statistical significance of expected result, in this case 1.96 at 95% confidence level ±5

p= prevalence of the disease (10%)

q= 1-p (1-0.10) = 0.90

d= desired precision level ±5 (0.05)

Therefore, n= 138.2976

Desired sample size = 150

3.5.3 Logistical and ethical considerations

i. Ethical clearance was given by Kenyatta National Hospital/University of Nairobi (KNH/UON) Ethics and Research Committee, Thika Level 5 (County) Hospital Ethics and Research Committee, where the study was conducted, as well as by the National Commission for Science, Technology and Innovation (NACOSTI).

ii. Participation by women in this study was voluntary. Thereafter, participants who volunteered to participate in the study and who met the inclusion criteria were

selected using simple random sampling method of Stat Trek's random number generator tool. They were taken through the informed consent form (ICF) and any questions they had clarified to them. Only those volunteers who voluntarily signed the ICF were recruited, with subsequent replacement of eligible participants who declined to sign the ICF.

iii. Confidentiality was maintained throughout the study. Participants only identified themselves by name when giving answers to the interviewer-administered questionnaire for purposes of the reports going to the patient's file. However, to ensure anonymity and confidentiality, accession numbers were given to each sample collected from the participants. This number was also placed on questionnaire and slides for each participant.

iv. During recruitment, women participants were informed about the study, the objectives to be achieved, beneficial and harmful effects of the study, procedures to be undertaken, results and their interpretation, treatment options and other follow-up measures for positive cases, eligibility criteria, period of the study, confidentiality of personal information and data obtained.

v. Every participant was required to give informed consent by signing or thumb printing on the Informed Consent Form.

vi. Any woman with abnormal test results was referred to the gynaecologist mentioned above for treatment and report sent to their clinical file.

vii. Each participant was informed of their individual results immediately after test results were finalized by a qualified gynaecologist who then provided clinical care.

3.6 Data collection methods

3.6.1 1nterviewer-administered questionnaire

This was used to capture socio-demographic information of each participant and was administered by the interviewer. It contained both open ended and closed ended questions and was divided into three sections; section one derived personal information such as age, marital status and educational level and last menstrual period (LMP); section two sought information on sexual lifestyle such as number of sexual partners, recent sexual activity, and section three sought information on relevant clinical history such as allergies, recent antibiotic therapy and reproductive

history on abnormal vaginal bleeding, knowledge of BV, intravaginal cleansing practices and pregnancy complications. 150 questionnaires were administered with 100% response rate since the interviewer clarified the difficult to understand questions to the participants.

3.6.2 Laboratory procedures

This involved four stages as follows;

3.6.2.1 Collection of vaginal samples

Vaginal smear was collected by a qualified nurse using cyto-pak Pap smear kit (tear fixative) (from IMEB Inc., San Marcos, California) consisting of cervical spatula, cervibrush, frosted glass slide and tear fixative for cytology. First, the patient was placed in lithotomy position; a sterile unlubricated vaginal speculum was inserted to visualize the vaginal canal. If any discharge was present, it was first noted and the colour also documented. A cervical spatula was then inserted into the vaginal canal to collect samples from the posterior fornix and lateral vaginal walls. In women who were pregnant or suspected to be pregnant, sampling was restricted to lateral vaginal wall.

3.6.2.2 Preparation of vaginal smear

Three smears were prepared from the collected vaginal sample from each participant. Collected material on the cervical spatula was spread immediately on the non-frosted surface of three pre-labelled frosted microscope slides to make vaginal smears, and if the material was not enough for smear preparation, resampling was repeated. Tear fixative was immediately applied on the smears on only two slides and further fixation was done by immersing the two slides in a coplin jar with 95% ethanol for 15 minutes. The smear on the third slide was air dried. Blinding of the smears was done by labelling the slides with unique codes that did not reveal the staining method to be used.

3.6.2.3 Staining of the prepared smears

The prepared smears were then stained using the appropriate method. Of the two fixed smears, one was stained with Conventional Papanicolaou method (RoyBiswas et al., 2008; Gachie et al., 2011) the second with Modified Papanicolaou (REAP) method (RoyBiswas et al., 2008) while the air-dried smears were exclusively stained with Gram stain method (Cheesbrough, 2006).

Table 3.1: Conventional Papanicolaou and Modified Papanicolaou (REAP) staining methods

Conventional Papanicolaou method		***Modified Papanicolaou (REAP) method***	
Fixation**:** 95% alcohol for 15 minutes.		Fixation**:** 95% alcohol for 15 minutes.	
Tap water	10 dips	1% acetic acid	10 dips
Harris Haematoxylin	10 dips	Harris's Haematoxylin ($60^{O}C$)	10 dips
Tap water	10 dips	Tap water	10 dips
95% ethanol	10 dips	1% acetic acid	10 dips
OG-6 stain	10 dips	OG-6 stain	10 dips
95% ethanol	10 dips	1% acetic acid	10 dips
EA 36/50	10 dips	EA 36/50	10 dips
95% ethanol	10 dips	1% acetic acid	10 dips
95% ethanol	10 dips	Absolute Methanol	10 dips
100% ethanol	10 dips	Absolute Methanol	10 dips
100% ethanol	10 dips	Absolute Methanol	10 dips
100% ethanol	10 dips	Xylene	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	Blotting after each step above	
Blotting after each step above		DPX mount and coverslip	
DPX mount and coverslip			

Table 3.2: Gram stain method

Step	Time
Heat fix air dried smear	
Crystal violet stain	1 minute
Tap water	
Gram's iodine	1 minute
Tap water	
Acetone-alcohol	6 seconds
Tap water	
Neutral red	2 minutes
Tap water	
Air dry	

3.6.2.4 Microscopic examination of the stained smears

The 2001 Bethseda system for reporting cervical cytology was used to screen and report the smears. The smear was considered adequate and satisfactory for evaluation when it had an estimated minimum of approximately 8,000 to 12,000 well preserved and well-visualized squamous epithelial cells (Solomon *et al.,* 2004; Nayar *et al.,* 2015). Reference images of known cellularity that simulate the appearance of conventional smears using 4× field were compared with the stained specimen by the microscopists to approximate the cellularity of the smears to be examined.

Light microscopy of the Conventional Papanicolaou and Modified Papanicolaou (REAP) stained vaginal smears was done using ×4, ×10 and ×40 objectives with presence of >20% clue cells being the threshold for a positive BV test. For Gram-stained vaginal smears, oil immersion objective (×100 objective) was used with a 7-10 Nugent score of bacterial morphotypes being positive for BV.

3.7 Quality assurance

Since laboratory diagnosis of BV was done through microscopic examination of stained vaginal smears, quality assurance and measures to minimize possible errors was achieved by ensuring that: the sample was collected from lateral vaginal wall and posterior fornix; preparation and reading of stained smears was in accordance with existing standard operating procedures (SOPs) (The 2001 Bethseda reporting guidelines for cervical cytology, 2001); there was proper maintenance and set up of microscopes: microscopists were competent and examination of the smears was first done by the principal investigator and confirmed by qualified microbiologist for

Gram stained smears and qualified cytologist for Conventional Papanicolaou and Modified Papanicolaou (REAP) stained smears. The cytologist was blinded to the Gram stain results. It is only at the end of this that the microscopists revealed their reports and any discrepancies confirmed by a second qualified microbiologist and second qualified cytologist in order to minimize intra- and interobserver variability

3.8 Data management

All collected data was double-entered into computer database in Microsoft Excel (Ms-Excel) computer application. To avoid loss or tampering, the document was password protected and back up of the data was done in compact discs, external hard disk and printing of the same data to have hard copies. The printed hard copies and duly filled questionnaires were filed in separately labelled box files which were kept in a secure lockable drawer away from physical and mechanical hazards and also to maintain privacy and confidentiality of information of the participating women.

Cleaning and validation of the data went on during data collection and entry in readiness for exportation of the data to IBM SPSS (Statistical Package for Social Sciences) version 20 for data analysis. Any additional information collected and observed during data collection was recorded on hard cover books and same kept in a lockable drawer for privacy, confidentiality, avoid loss and tampering of the information.

3.9 Data analysis

This was done using IBM SPSS (Statistical Package for Social Sciences) version 20 at 95% confidence interval and statistically significant p value of ≤ 0.05 according to the two specific objectives and two research questions that guided this study and data collection.

3.9.1 Screening of BV in vaginal smears using Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain methods

A frequency distribution bar graph indicating the number of positive and negative cases of BV was used to show difference in screening of BV in vaginal smears stained using three different staining methods-Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain.

3.9.2 Sensitivity, specificity, PPV and NPV of Modified Papanicolaou (REAP) and Conventional Papanicolaou methods

The diagnostic efficiency statistical measures of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio and diagnostic accuracy were selected to establish if Modified Papanicolaou (REAP) was a suitable alternative to Conventional Papanicolaou method in screening of BV in vaginal smears. This is because these diagnostic efficiency statistics are objective measures that evaluate the discriminative and predictive abilities of a diagnostic screening test. Additional data analysis statistical measures used are false positive rate (FPR), false negative rate (FNR), likelihood ratios (LR) for positive test (LR+) and negative test (LR-), overall diagnostic accuracy (DOR), ROC curve analysis and kappa statistic measure.

3.10 Operational definition of terms

The following definitions are provided to ensure uniformity and understanding of these terms throughout the study.

1. **Sensitivity-** shows percentage of people with the disease (a) at the time of screening who will have a positive test result (a+c) (true positives/ (true positives + false negatives); a/(a+c) (Glick, 2009; Stojanovic, 2014). It is also known as true positive rate (TPR) i.e., positive in disease.

It is calculated using the formula:

$$\text{Sensitivity} = \frac{\text{True positives (TP)}}{\text{True positives (TP) + False negatives (FN)}} \quad \text{OR} \quad \frac{a}{a+c}$$

2. **Specificity-**shows percentage of people not having the disease (d) at the time of screening who will have a negative test result (true negatives/ (true negatives + false positives); d/(b+d) (Glick, 2009; Stojanovic, 2014). It is also known as true negative rate (TNR) i.e. negative in health.

It is calculated using the formula:

$$\text{Specificity} = \frac{\text{True negatives (TN)}}{\text{True negatives (TN) + False positives (FP)}} \quad \text{OR} \quad \frac{d}{b+d}$$

3. Positive predictive value (PPV)- shows proportion of people with a positive test result (a) who are actually ill (a + b) at the time of screening i.e., proportion of disease given a positive test (Glick, 2009; Stojanovic, 2014).

It is calculated using the formula:

$$PPV = \frac{\text{True positive (TP)}}{\text{True positive (TP) + false positive (FP)}} \quad OR \quad \frac{a}{a+b}$$

4. Negative predictive value (NPV)- shows proportion of people with a negative test result (d) who actually do not have the disease (b + c) at the time of screening i.e. proportion of no disease given a negative test (Glick, 2009).

It is calculated using the formula:

$$NPV = \frac{\text{True negative (TN)}}{\text{True negative (TN) + false negative (FN)}} \quad OR \quad \frac{d}{c+d}$$

5. False positive rate (FPR)- number of false positive test results for an outcome (b) divided by the total number of absence of an outcome (b+d)

It is calculated using the formula:

$$FPR = \frac{c}{c+d}$$

6. False negative rate (FNR)- number of false negative test results for an outcome (c) divided by the total number of presence of an outcome (a+c)

It is calculated using the formula:

$$FNR = \frac{b}{a+b}$$

7. Likelihood ratios-shows probability of a specific test result being found in a person who has the condition of interest at the time of screening to the probability that the same specific test result would be found in a person who does not have the condition of interest at the time of screening. There are two likelihood ratios, one for positive test results, (LR+), known as the positive likelihood ratio and another for negative test results (LR-), known as negative likelihood ratio. LR value greater than 1 (common in LR+) for a specific test result indicates that test result is associated with the presence of the disease, in most cases. On the other hand, LR value less than 1 (common in (LR-) for a specific test result indicates that that test result is associated with the absence of disease (Wikipedia, 2021).

They are calculated using the formula:

$$LR + = \frac{\text{Sensitivity}}{(1 - \text{Specificity})}$$

$$LR - = \frac{(1 - \text{Sensitivity})}{\text{Specificity}}$$

The above diagnostic efficiency statistic measures can be explained using a 2x2 contingency table approach that categorizes diagnostic test results as positive or negative.

Table 3.3: 2×2 Contingency table

	Reality		
Test result	*Disease present (n)*	*No disease present (n)*	**Totals (n)**
Positive	True positive (TP)	False positive (FP)	
	A	B	a+b
Negative	False negative (FN)	True negative (TN)	
	C	D	c+d
Totals	a+c	b+d	a+b+c+d
	(All with disease)	(All normal)	

[Source: Shaughnessy Allen F. Clinical Information Sciences]

[a – true positive (sick people correctly diagnosed as sick); b – false positive (healthy people wrongly diagnosed as sick); c – false negative (sick people wrongly diagnosed as healthy); d – true negative (healthy people correctly diagnosed as healthy)]

3.11 Limitations and delimitations

3.11.1 Limitations

Limitations are factors usually beyond the researcher's control that may have been impossible to avoid or minimize, and that may affect the results of the study or how the results are interpreted, (Murnan & Price, 2004). Due to limited resources and time in conducting this research, this study faced several limitations. The major limitation was that during the study period, the study participants were recruited from only one hospital and this may not be representative of the annual female population

served by the hospital. In addition, only women visiting ante-natal clinic (ANC) and family planning (FP) clinics were recruited into the study and the results may not be applicable to women delivering at the hospital, hence, this will affect the generalizability of the findings. However, this will be augmented when the research can be applied to other populations of women.

3.11.2 Delimitation

Delimitation refers to boundaries set by the researcher in order to limit the scope of the findings (Simon, 2011). In conducting this research, this study had several delimitations. First, regarding geographical delimitation, this study was limited to Kiambu County, Kenya, whereby only one hospital, Thika level 5 (County) Hospital was used in recruitment of the study subjects. This was due to limited resources and time. Secondly, all females aged 18-45 years and who gave consent were eligible for the study but exclusion was based on use of any antibiotic and/or vaginal creams in the last two weeks prior to start of the study; presence of active vaginal bleeding and engagement in sexual intercourse within the last 8hrs at the time of recruitment. This exclusion was due to the potential of exclusion factors to cause disturbance of bacterial microbial flora. Lastly, this study restricted diagnosis of BV to presence/absence of clue cells and did not perform Nugent scoring for the screening methods used due to extensive training required to train smear readers on scoring.

3.12 Assumptions

Assumption refers to a statement that is presumed to be true by other scholars but has not been proved scientifically (Simon, 2011). The following assumptions were made regarding this study. First, the instrument to be used would elicit reliable responses, second, the respondents would fully understand the questions they would be asked, third, the participants would answer the interview questions in an honest and candid manner, fourth, the inclusion criteria of the sample was appropriate and ensured homogeneity of research experience for all the participants and lastly, that there was no ill motive on the part of the participants in participating in the research.

CHAPTER FOUR

RESULTS

Table 4.1: Socio-demographic characteristics of the participants

Epidemiological variable	Outcome	Number, n=150 (Percentage)
Age	18-25 years	83(55.3%)
	26-35 years	47(31.3%)
	36-45 years	20(13.3%)
Marital status	Single	49(32.7%)
	Married	101(67.3%)
Education	Primary	49(32.7%)
	Secondary	79(52.7%)
	Tertiary	22(14.6%)
Socio-economic status	Employed	68(45.3%)
	Un-employed	82(54.7%)
Sexual history	Multiple sexual partners	22(14.7%)
	One sexual partner	108 (72%)
	No sexual partner	20 (13,3%)
Overview of BV	Knowledge of BV	21(14%)
	Previous BV test	10(6.7%)
	No knowledge of BV and no previous BV test	119 (79.3%)
Clinical and reproductive history	History of abnormal vaginal bleeding	2(1.3%)
	Previous pregnancy complications(miscarriage)	4(2.7%)
	No history of abnormal vaginal bleeding and no previous pregnancy complications	144 (96%)
Practice of intravaginal practices (e.g. douching, vaginal creams, finger cleansing)	Yes	5(3.3%)
	No	145 (96.7%)
Vaginal discharge	Present	33(22%)
	White colour	33(22%)
	Odour	19(57.6%)
	Thin watery	33(22%)
	Absent	32 (21.3%)
Last menstrual period (LMP)	Suspected pregnant participants	2 (1.3%)
	Possibility of non-pregnant participants	148 (98.7%)
Antibiotic use	Yes	
	- 3 weeks before sampling	2 (1.3%)
	- 1 month before sampling	20 (13.3%)
	- 4-6 months before sampling	18 (12%)
	No	110 (73.3%)

Demographics show that majority of the participants were married, literate with secondary level of education and largely unemployed. Relatively low number

reported multiple sexual partners, knowledge of BV, practice of intravaginal cleansing practises, history of abnormal vaginal bleeding and previous pregnancy complications. On pelvic examination, 22% (33/150) of the female participants presented with a white, thin, watery vaginal discharge that was predominantly odourless.

4.1 Results of age distribution of the women screened for bacterial vaginosis

Table 4.2: Age distribution of the women screened for bacterial vaginosis

Age group	Number of participants	Percentage
18-25 years	83	55.3%
26-35 years	47	31.3%
36-45 years	20	13.3%
Total	**150**	**100%**

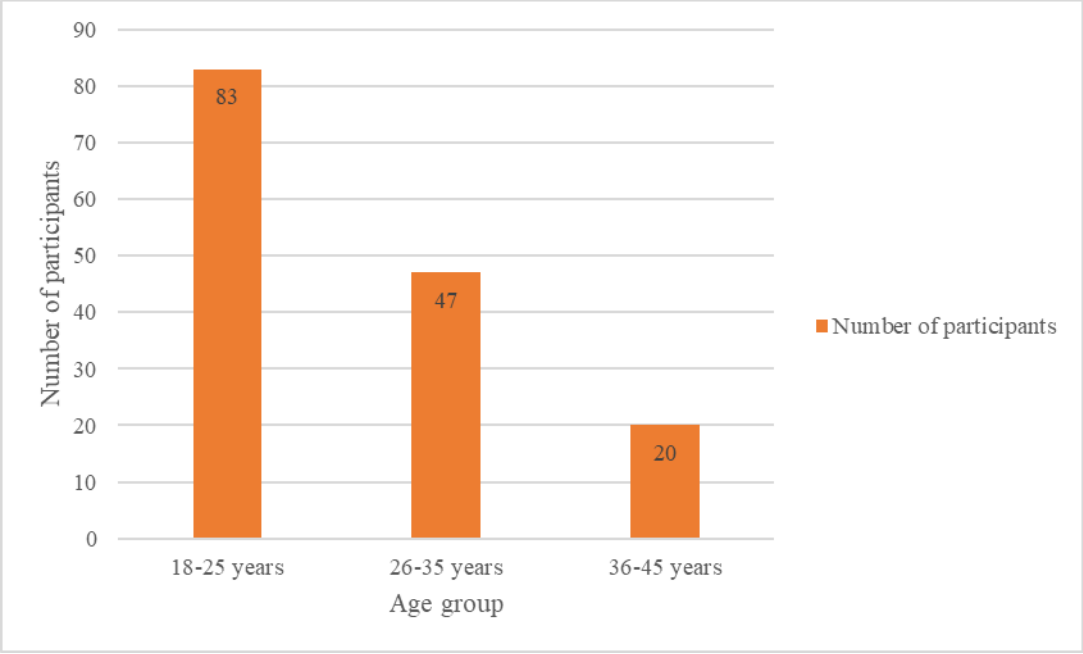
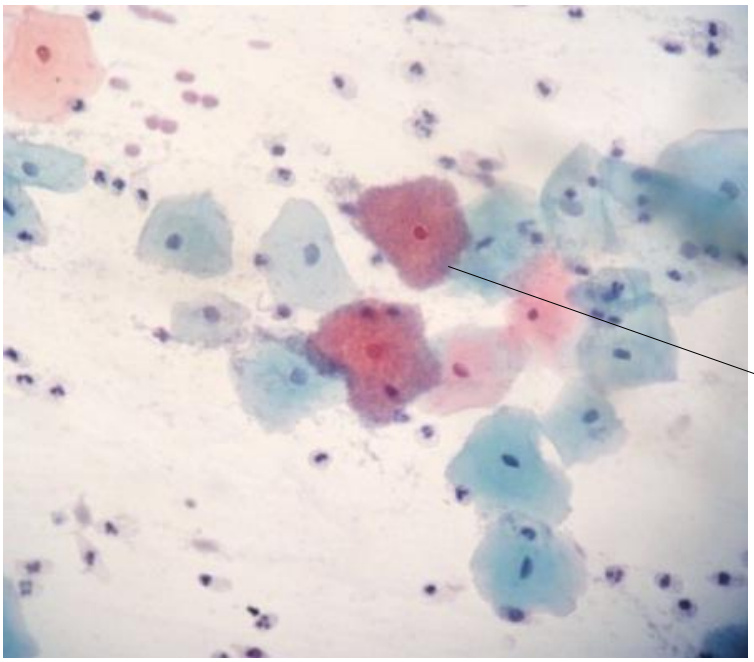


Figure 4.1: Age distribution of the women screened for bacterial vaginosis

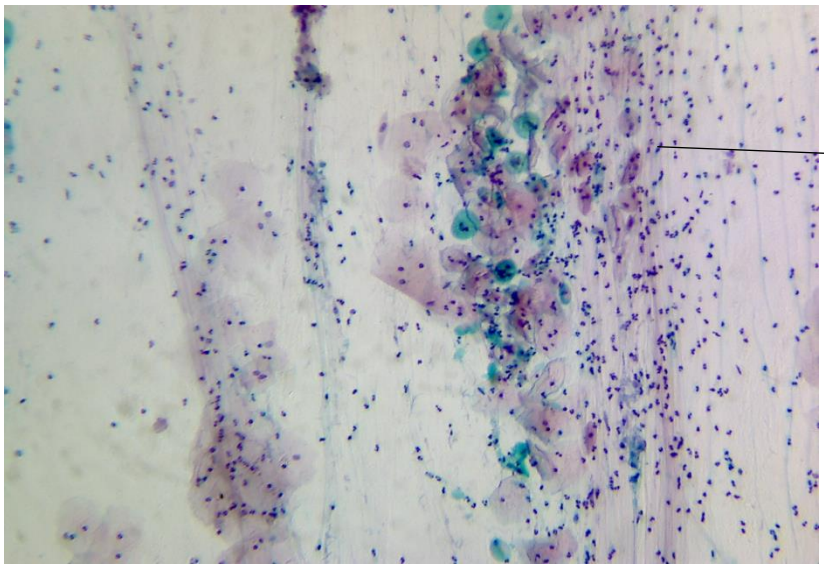
The mean age of the study participants was 26.9 years, median of 25, standard deviation (SD) of 5.9 with minimum and maximum ages of 19 and 42 years respectively. Of the 150 participants, 55.3% were 18-25 years old, 31.3% were 26-35 years while the least number, 13.3% were 36-45 years old.

4.2 Results of screening of BV in vaginal smears using Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain methods

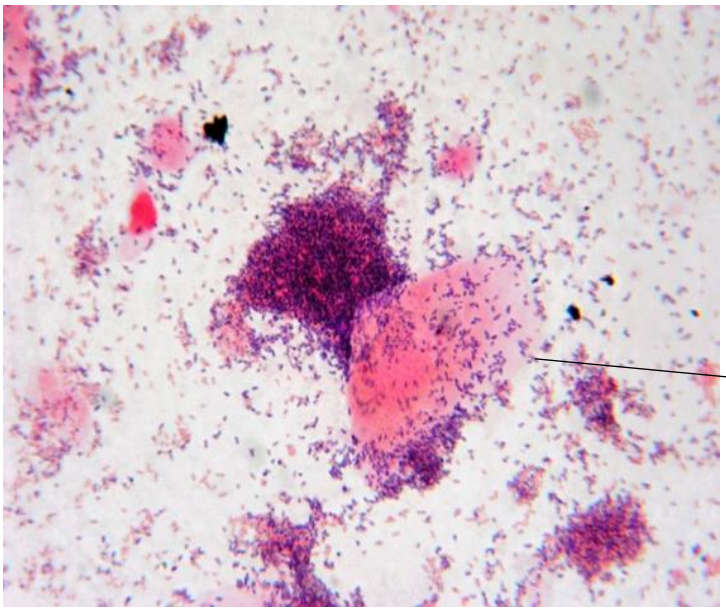
Out of 150 paired vaginal smears examined in the study, 145 pairs were found satisfactory for evaluation. 5 pairs of smears were excluded as they were unsatisfactory for evaluation due to scanty cellularity and a repeat was recommended and performed. All the smears were negative for intraepithelial lesion but 25 and 16 smears stained with Conventional Papanicolaou and Modified Papanicolaou (REAP) methods were suggestive of bacterial vaginosis.



Slide 1: Vaginal smear stained using Conventional Papanicolaou method (×40 objective)



Slide 2: Vaginal smear stained using Modified Papanicolaou (REAP) method (×10 objective)



Slide 3: Gram-stained vaginal smear showing bacterial vaginosis (x10 objective)

Table 4.3: Conventional Papanicolaou * Modified Papanicolaou (REAP) method *Gram stain method Crosstabulation

		Gram stain method		
		Positive	**Negative**	**Total**
Conventional Papanicolaou method	Positive	20	5	25
	Negative	22	103	125
Modified Papanicolaou (REAP) method	Positive	11	5	16
	Negative	31	103	134
	Total count	**42**	**108**	**150**

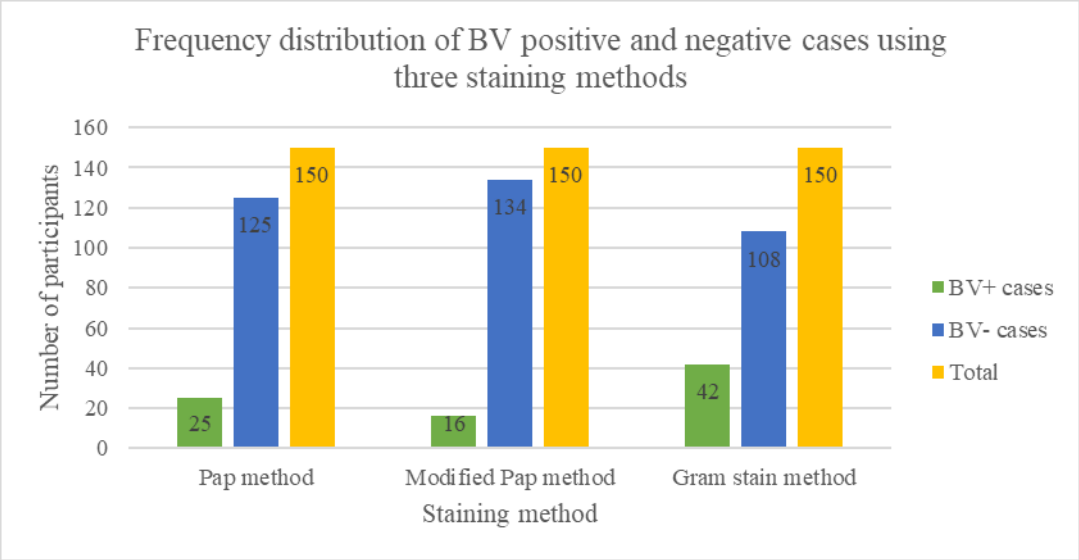


Figure 4.2: Screening of bacterial vaginosis using Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain methods

Gram stain method Nugent's scoring system which was the diagnostic gold standard in this study detected 42 positive cases of bacterial vaginosis out of 150 (42/150) participants, representing a prevalence of 28%, with majority of the cases (20/42) found in the 26-35 years age group. Six participants (6/150) had intermediate flora and were considered negative for bacterial vaginosis. On the other hand, 25 participants (25/150) representing 16.7% and 16 participants (16/150) representing 10.7% tested positive for bacterial vaginosis using Conventional Papanicolaou and Modified Papanicolaou (REAP) methods respectively. A total of 125 participants (125/150) representing 83.3% and 134 participants (134/150) representing 89.3% tested negative for bacterial vaginosis using Conventional Papanicolaou and Modified Papanicolaou (REAP) methods respectively.

The results indicate that there is a difference in diagnosis of bacterial vaginosis (BV) in vaginal smears between the three staining methods of Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram stain.

4.3 Results of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of ConventionalPapanicolaou and Modified Papanicolaou (REAP)methods

Table 4.4: Conventional Papanicolaou method * Gram stain method Crosstabulation

			Gram stain method		Total
			Positive	Negative	
Conventional Papanicolaou method	Positive	Count	20	5	25
		% within Conventional Papanicolaou method	80.0%	20.0%	100.0%
		% within Gram stain method	47.6%	4.6%	16.7%
	Negative	Count	22	103	125
		% within Conventional Papanicolaou method	17.6%	82.4%	100.0%
		% within Gram stain method	52.4%	95.4%	83.3%
Total		Count	42	108	150
		% within Conventional Papanicolaou method	28.0%	72.0%	100.0%
		% within Gram stain method	100.0%	100.0%	100.0%

Using Gram stain as the reference gold standard test in this study, the diagnostic efficiency statistical measures of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for Conventional Papanicolaou method was determined at 95% confidence interval and statistically significant p value of ≤ 0.05.

Conventional Papanicolaou method had sensitivity of 47.6% with 20 out of 42 (20/42) participants testing positive for BV and specificity of 95.4% with 103 out of 108 (103/108) participants testing negative for BV. 22 participants who were BV positive using Gram stain Nugent's scoring system but tested BV Conventional

Papanicolaou method gave a false negative rate (FNR) of 52.4% while 5 participants who were BV negative using Gram stain Nugent's scoring system but tested BV positive using Conventional Papanicolaou method gave a false positive rate (FPR) of 4.6%. Positive predictive value (PPV) was 80.0% while the negative predictive value (NPV) was 82.4%. The likelihood ratio for a positive test (LR+) was 10.3 while likelihood ratio for a negative test was 0.55. The overall diagnostic accuracy was 38.9%.

4.4 Sensitivity, Specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Modified Papanicolaou (REAP)method with reference to Gram stain method as the gold standard in screening of BV

Table 4.5: Modified Papanicolaou (REAP)method * Gram stain method Crosstabulation

			Gram stain method		Total
			Positive	Negative	
Modified Papanicolaou method	Positive	Count	11	5	16
		% within Modified Papanicolaou method	68.8%	31.2%	100.0%
		% within Gram stain method	26.2%	4.6%	10.7%
	Negative	Count	31	103	134
		% within Modified Papanicolaou method	23.1%	76.9%	100.0%
		% within Gram stain method	73.8%	95.4%	89.3%
Total		Count	42	108	150
		% within Modified Papanicolaou method	28.0%	72.0%	100.0%
		% within Gram stain method	100.0%	100.0%	100.0%

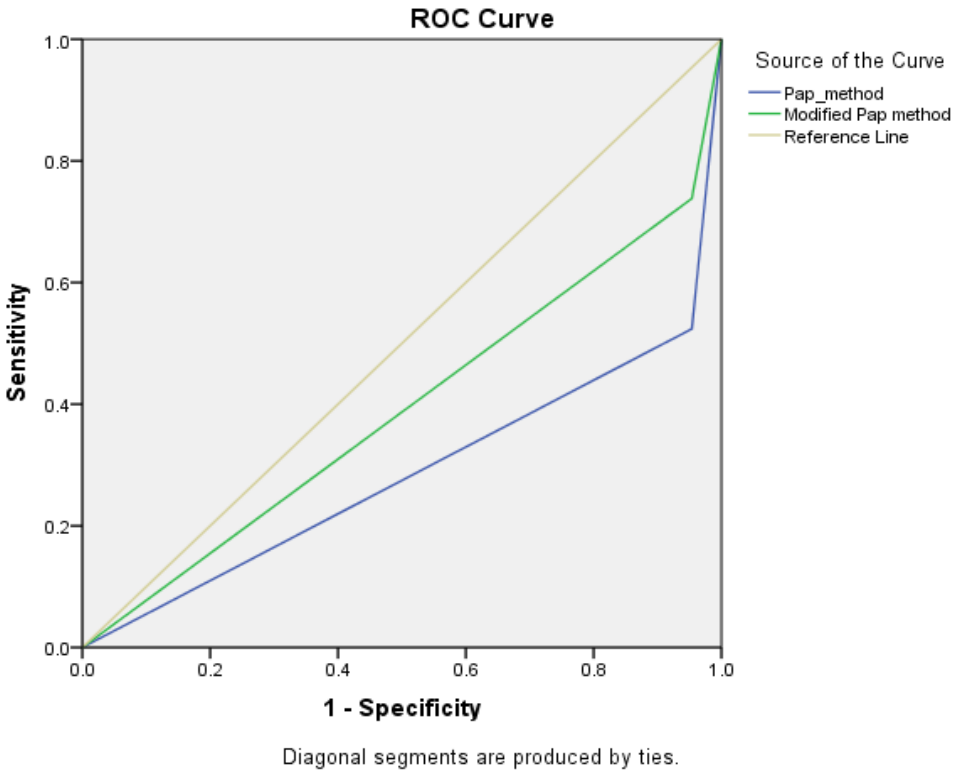
Using Gram stain as the reference gold standard test in this study, the diagnostic efficiency statistical measures of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for Modified Conventional Papanicolaou (REAP) method was determined at 95% confidence interval and statistically significant p value of ≤ 0.05.

Modified Papanicolaou (REAP) method had sensitivity of 26.2% with 11 out of 42 (11/42) participants testing positive for BV and specificity of 95.4% with 103 out of 108 (103/108) participants testing negative for BV. 31 participants who were BV positive using Gram stain Nugent's scoring system but tested BV Modified Papanicolaou (REAP) method gave a false negative rate (FNR) of 73.8% while 5 participants who were BV negative using Gram stain Nugent's scoring system but tested BV positive using Modified Papanicolaou (REAP) method gave a false positive rate (FPR) of 4.6%. Positive predictive value (PPV) was 68.8% while the negative predictive value (NPV) was 76.9%. The likelihood ratio for a positive test (LR+) was 5.696 while likelihood ratio for a negative test was 0.77. The overall diagnostic accuracy was 38.9%.

The results show that Conventional Papanicolaou and Modified Papanicolaou (REAP) methods have varying diagnostic efficiency statistical measures of sensitivity, positive predictive value (PPV) and negative predictive value (NPV) but same specificity. However, both methods had the same overall diagnostic accuracy which indicates that Modified Papanicolaou (REAP) method can be a suitable alternative to Conventional Papanicolaou method especially in confirming truly negative BV cases.

4.5 Receiver Operating Characteristic (ROC) curve of Conventional Papanicolaou method and Modified Papanicolaou (REAP) method

Using Gram stain as the reference gold standard test in this study, the diagnostic efficiency statistical measures of sensitivity and specificity of Conventional Papanicolaou and Modified Papanicolaou (REAP) methods were compared using ROC curve at 95% confidence interval and statistically significant p value of 0.05. The results are indicated in Figure 4.3.



Area Under the Curve

Test Result Variable(s)	Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Conventional Papanicolaou method	.285	.053	.000	.182	.388
Modified Papanicolaou method	.392	.055	.041	.285	.500

The test result variable(s): Conventional Papanicolaou method, Modified Papanicolaou method has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

Figure 4.3: ROC curve of Conventional Papanicolaou and Modified Papanicolaou (REAP) methods

With respect to the reference line which represents the reference gold standard test used in this study, that is, Gram stain Nugent scoring system, the results show that

the ROC curves for both Conventional Papanicolaou and Modified Papanicolaou (REAP) methods fall below the reference line.

The area under the curve (AUC) values were 0.285 (p=.000, 95% CI-.182-.388) and 0.392 (p=.041, 95% CI-.285-.500) for Conventional Papanicolaou and Modified Papanicolaou (REAP) methods respectively and statistically significant ($p \leq 0.05$).

The results indicate that the two methods have very poor sensitivity but high specificity compared to Gram stain method in screening of BV cases.

4.6 Cohen Kappa statistic value of Conventional Papanicolaou and Modified Papanicolaou (REAP) methods

Table 4.6: Modified Papanicolaou (REAP) method * Conventional Papanicolaou method Crosstabulation

Symmetric Measures

		Value	Asymp. Error[a]	Std.	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.692	.086		8.753	.000
N of Valid Cases		150				

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

A statistically significant ($p \leq 0.05$) kappa value of 0.692 was obtained representing moderate agreement in the diagnostic efficiency capabilities of Conventional Papanicolaou and Modified Papanicolaou (REAP) methods in screening of bacterial vaginosis in vaginal smears.

The results indicate that Modified Papanicolaou (REAP) method can be a suitable alternative to Conventional Papanicolaou method in screening of bacterial vaginosis in vaginal smears.

CHAPTER FIVE

DISCUSSION

Bacterial vaginosis (BV) is a polymicrobial syndrome characterized by shift in vaginal flora (Demba et al., 2005; Togni et al., 2011) with replacement of the predominant and naturally occurring *Lactobacilli* species with mostly facultative anaerobic bacteria consisting of *Gardnerella vaginalis, Bacteroides spp., Mobiluncus spp., Prevotella spp. Atopobium vagina* and *Mycoplasma hominis* (Biswal et al., 2014). It is common in women in the reproductive age group (Patterson et al., 2010). Most of the women are asymptomatic but symptoms include a thin, white, watery discharge, vaginal pH >4.5, positive amine 'whiff' test on addition of 10% potassium hydroxide and presence of clue cells on wet microscopy of vaginal fluid (Neelam et al., 2010). BV has been associated with adverse and serious reproductive health outcomes (Sweet, 2000), CIN development (Nam et al., 2009) and HIV infectivity (Woodman, 2016).

Mean age was 26.9, median of 25 and standard deviation (SD) of 5.9. This is comparable to a study done by Shayo et al. (2012) in Mwanza, Tanzania where median age of the participants was 26 years. 101 (67.3%) of the participants were married and 49 (32.7%) were single. This is different from a study done by Nzomo et al. (2013) in Thika, Kenya that had 40.9% of the women married and 47.2% single. This difference could be due to the study design that sampled participants once and this may have captured women with varied demographic characteristics. Among the study participants, 52.7% and 14.6% had secondary and tertiary education respectively and this is comparable to 52.3% and 17.1% reported in a study in Thika by Nzomo et al. (2013). The difference could be due to the difference in the sample size in the two studies. The highest number of BV cases, 47.6% was in the 26-35 years age group and this is similar to the study by Nzomo et al. (2013) which had 47.7% but different in age group due to varied demographic categorization.

An estimated 25-30% of women have asymptomatic BV at any given time and this may be as high as 85% in female sex workers (FSW) (Eifediyi et al., 2015) with a prevalence of 20-50% in African women (Sobel, 2000) and 55% in women in sub-Saharan Africa (Woodman, 2016). A study in Kenya by Bukusi et al. (2006) reported a 44% prevalence rate while Nzomo reported 26% and 43.1% in 2011 and 2013

respectively in women in Thika. In a respondent driven sampling study among female sex workers (FSW) in Nairobi Kenya, Musyoki et al. (2015) reported BV prevalence of 15.1%. This is slightly different from a study on FSW in Kisumu, Western Kenya by Vandenhoudt et al. (2013) that reported a 27.0% BV prevalence. In a cross-sectional study among pregnant women in a rural county hospital in Kilifi, BV prevalence was 19.3% (Masha et al., 2017). A longitudinal study conducted in Kenya, Rwanda and South-Africa by Jespers et al. (2014) reported BV prevalence of 38% in women at the screening visit. In the present study, BV was detected in 28% of the women and this is similar to a study in Ghana by Aubyn et al. (2013) that reported BV prevalence of 28%. The age group of 26-35 years had the most BV cases with a prevalence of 47.6% which is different from 31-40 years age group with a prevalence of 68.69% reported by Narasimha et al. (2014) in a retrospective study on married women. This difference may be attributed to the use of only married women in the study.

A good diagnostic test should have high sensitivity and high specificity while a good screening tool should be able to identify individuals from an asymptomatic population with a disease/condition of interest. BV is commonly diagnosed clinically using Amsel's composite criteria or by laboratory-based Nugent scoring of Gram-stained vaginal smears (Rao et al., 2016; Mahajan et al., 2017). Amsel's criteria requires presence of three out of four of the following; (1) vaginal discharge that is homogeneous and milky, (2) vaginal pH greater than 4.5; (3) a positive whiff test (a fishy amine odor on addition of 10% KOH on vaginal fluid) and (4) clue cells on a saline wet mount of vaginal fluid (Neelam et al., 2010). This study reports white vaginal discharge in 33(22%) of the participants which is lower than 79(68.69%) and higher than the 17% reported by Narasimha et al. (2014) and Vardar et al. (2002) respectively. This could be attributed to the different sample sizes used in the studies. Nugent's scoring of Gram-stained vaginal smears is considered the gold standard in diagnosis of BV (Nugent et al., 1991). Other proposed criteria for BV diagnosis is presence of clue cells in Pap smears (Sachdeva, 2006; Filho *et al.*, 2010; Truter et al., 2013) with a threshold of >20% clue cells considered diagnostic of BV (Discacciati et al., 2006).

Many authors have reported varied results on the performance of Pap smear in the screening of BV using Gram stain as the reference gold standard. In two prospective studies on women, one in Mombasa, Kenya by Karani et al. (2007) and another by Platz-christensen et al. (1995), sensitivity, specificity, PPV and NPV values were 59.4%, 83.3%, 67.3%, 78.0% and 88.2%, 98.6%, 96.8%, 94.7% respectively. In another study on non-pregnant women, Fan et al. (1996) reported sensitivity of 85.1% and specificity 95.1%. Additionally, a prospective study by Tokyol et al. (2004) reported sensitivity of 43.1%, specificity of 93.6%, PPV of 73.8% and NPV of 79.8%. In different studies on pregnant women, Lamont et al. (1999) reported sensitivity and specificity of 80.7% and 90.7% respectively while Bombase et al. (2014) reported sensitivity, specificity, PPV and NPV of 70.45%, 93.56%, 80.52% and 89.43% respectively. All these authors utilized Pap-stained cervical smears and only Platz-christensen et al. (1995) used Pap-stained vaginal smears in their studies.

Livengood (2009) reported that Pap test has sensitivity as low as 50% and specificity of about 95% in diagnosis of BV indicating that a positive result is reliable evidence of BV presence but a negative result does not rule out presence of BV. This present study utilized Gram stain method as the reference diagnostic standard in evaluating diagnostic performance of Conventional Papanicolaou and Modified Papanicolaou (REAP) methods and reports sensitivity of 47.6% and 26.2%, specificity of 95.4% and 95.4%, PPV of 80% and 68.8% and NPV of 82.4% and 76.9% for Conventional Papanicolaou and Modified Papanicolaou (REAP) methods respectively. The results for Conventional Papanicolaou method are comparable to those reported by Platz-Christensen et al. (1995). The varied results may be attributed to interobserver variability with utilization of many cytologists resulting in lower sensitivity as opposed to a single cytologist. Other contributing factors may be expertise of the cytologist reporting the smears, type of population used (whether pregnant or non-pregnant subjects), environment and other socio-demographic characteristics of the participants, research design and specimen source site (cervix/endocervix as opposed to posterior fornix and lateral vaginal wall). However, a common finding of the above studies and which is reflected in this present study is the high specificity and low sensitivity for both Conventional Papanicolaou and Modified Papanicolaou (REAP) methods. Hodiwala et al. (2015) attributed this to lack of strict application of

standardized criteria for evaluation of Pap smears. This finding is further supported by this study's ROC analysis that shows statistically significant area under the curve (AUC) of 0.285and 0.392 for Conventional Papanicolaou and Modified Papanicolaou (REAP) methods respectively (p values of 0.000 and 0.041). The AUCs indicate that with reference to Gram stain method, both these methods are less sensitive in detecting BV cases hence are not suitable if Gram stain method is available but have diagnostic value (Filho et al., 2010; Bombase et al., 2014; Siddig et al., 2017). This implies that a BV-positive result is a strong evidence that the disease is present, while a BV-negative result does not conclusively indicate absence of BV (Livengood, 2009). In this study, a kappa value of 0.692 showed moderate agreement between Conventional Papanicolaou and Modified Papanicolaou (REAP) methods with the overall diagnostic accuracy of 38.9% for both methods. Additionally, Filho et al. (2010) reported that Pap method would be a valid diagnostic option in comparison to gold standard when it especially gives a positive BV result and a mean specificity of 95%. Since Modified Papanicolaou (REAP) has also fulfilled the condition by Filho et al. (2010), this study supports the use of Modified Papanicolaou (REAP) as a suitable alternative to Conventional Papanicolaou method in the absence of the gold standard.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study sought to establish if Modified Papanicolaou (REAP) method was a suitable alternative to Conventional Papanicolaou method in screening of BV in vaginal smears. In this endeavour, this study faced several limitations that will affect the generalizability of the results. First, due to limited resources and time in conducting this research, the research subjects were recruited from only one hospital during the study period and this may not be representative of the annual female population served by the hospital. Secondly, only women visiting ante-natal clinic (ANC) and family planning (FP) clinics were recruited and the results may not be applicable to women delivering at the hospital hence affecting the generalizability of the findings but this will be augmented when the research can be applied to other populations of women. Thirdly, due to recall or social desirability bias, self-reported information may have been misreported or under-reported during the questionnaire interview and lastly, extensive training requirements made it impossible to perform Nugent scoring of Modified Papanicolaou (REAP) and Conventional Papanicolaou methods.

In spite of these limitations, the greatest strength of this study is that it showed that Modified Pap has diagnostic value for BV diagnosis when it is positive and is therefore suitable as a confirmatory test for BV. This study supports the use of Modified Papanicolaou (REAP) as an alternative to Conventional Papanicolaou method in screening of BV in vaginal smears in the absence of the gold standard.

6.2 Recommendations

6.2.1 Policy recommendations

This study recommends addition of specimen sourcing sites of posterior fornix and lateral vaginal wall to routine Pap smear screening protocol for diagnosis of BV to avoid repeat sampling for microbiological analysis of BV and avoid duplication of tests.

6.2.2 Recommendations for further research

This study recommends further research to be done on Nugent scoring of Modified Papanicolaou (REAP) stained vaginal smears to build up on a stronger overall

evidence base on the use of Modified Papanicolaou (REAP) method as a suitable alternative to Conventional Papanicolaou method in screening of BV in vaginal smears.

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APPENDICES

Appendix 1: Informed Consent Form

This Informed Consent Form is for women who are attending Family planning and ante-natal clinic at Thika District Hospital, and who I am inviting to participate in research on Bacterial vaginosis. The title of my research project is "Comparison of Conventional Papanicolaou, Modified Papanicolaou (REAP) and Gram-stained vaginal smears in the screening of BV in women attending Thika Level 5 (County) Hospital".

Name of Principal Investigator : Grace Chepkemoi Manyu

Name of Organization : Jomo Kenyatta University of Agriculture and Technology

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with you)**
- **Certificate of Consent (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form.

PART I: CLIENT CONSENT INFORMATION

Introduction

My name is Grace Chepkemoi Manyu, a student pursuing a degree in Master of Medical Laboratory Sciences (Clinical Histopathology & Diagnostic cytology option) at Jomo Kenyatta University of Agriculture and Technology (JKUAT). I am conducting research on Bacterial vaginosis, which is a disease of public health concern in Kenya. I am going to give you information and invite you to be part of this research. You do not have to decide today whether or not you will participate in the research. Before you decide, you can talk to anyone you feel comfortable with about the research.

There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain. If you have questions later, you can ask them of me, the study doctor or the staff.

Purpose of the research

Bacterial vaginosis is a vaginal infection that causes a lot of discomfort to women and is associated with various complications in pregnant and non-pregnant women which if not treated, can lead to potentially fatal diseases. This study aims at

comparing three methods in the diagnosis of Bacterial vaginosis in women attending Thika Level 5 (County) Hospital.

Type of Research Intervention

This research will involve collection of vaginal sample only once from you. The sample will be tested in the laboratory and the results will be communicated to you. Should you require treatment after the results, this will also be communicated to you, and the doctor in the clinic you are now attending will discuss them with you in addition to the appropriate treatment.

Participant selection

I am inviting all female adults aged 18-45 years who are attending family planning and ante-natal clinic at Thika Level 5 (County) Hospital to participate in this research.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. Whether you choose to participate or not, all the services you receive at this clinic will continue and nothing will change. If you choose not to participate in this research project, you will be offered the treatment that is routinely offered in this clinic for bacterial vaginosis. You may change your mind later and stop participating even if you agreed earlier. You will not pay anything to participate in this research; neither will you receive any payment for your participation. You may not have any direct benefit at this moment but the information gained from this research will help in the future.

Procedures and Protocol

A. Unfamiliar Procedures and Description of the Process

The doctor will explain the procedure to you and collect vaginal sample only once. If you have any questions about anything, please feel free to ask.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected during the research will be put away and no-one but the researchers will be able to see it. Any information about you will have a number on it instead of your name. Only the researchers will know what your

number is and we will lock that information up with a lock and key. It will not be shared with or given to anyone except the doctor.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment at this clinic in any way. You will still have all the benefits that you would otherwise have at this clinic. You may stop participating in the research at any time that you wish without losing any of your rights as a patient here. Your treatment at this clinic will not be affected in any way. It is your choice and all of your rights will still be respected.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact any of the following: KNH/UoN ERC or Thika Level 5 (County) Hospital.

This proposal has been reviewed and approved by KNH/UoN ERC, which is a committee whose task it is to make sure that research participants are protected from harm. If you wish to find out more about this research, please contact KNH/UoN ERC on the following contacts:

Telephone number :- +2542726300-19 Ext.44102

E-mail:uonknh_erc@uonbi.ac.ke

Post address: P O BOX 20723-00202, Nairobi, Kenya

Physical Location:

Kenyatta National Hospital is located along the Hospital Road(off Ngong Road). On the northern side, it faces Ngong Road near its roundabout with Mbagathi Road. On the eastern side is Hospital Road to the West is Mbagathi Road. To the south of the hospital compound is the Nairobi-Kisumu railway line.

The KNH/UoN-ERC secretariat is located at the School of Pharmacy, UoN behind the KNH Dental clinic.

Kenyatta National Hospital is about 4 km from the General Post Office (GPO) and one can reach the secretariat by using bus route No.7C.

PART II: Informed Consent Form

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been

answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant :_____

Signature of Participant :_____

Date_____

Day/month/year

If participant is not able to read

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness_____ AND Thumbprint of participant

Signature of witness_____

Date_____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands what will be done in the research.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Name of person taking consent: Dr. John Njoroge (Gynecologist, Thika Level 5 (County) Hospital.

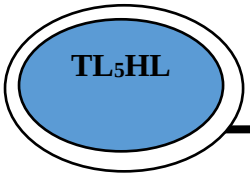
Signature of person taking consent: _____

Date_____

Day/month/year

Appendix 2: Laboratory request and report form

TL5HLQMSF053



THIKA LEVEL 5 HOSPITAL LABORATORY

Dedicated to provide our customers with timely and efficient services

LABORATORY REQUEST AND REPORT FORM

Note: incompletely filled forms will not be processed

I. Patient details Name…… Age (yrs/months)……. Sex M ☐ ☐ Residence/Village…….. IP/OP No……	**II. Specimen destination** Tick appropriate box Histology/Cytology ☐ Bacteriology ☐ Serology ☐ Parasitology ☐ Haematology ☐ Biochemistry ☐
III. Previous Report…… **Previous Lab No**……..	Sputum New ☐ Follow up ☐ 1st ☐ 2nd ☐ 3rd ☐
IV. Specimen..... Collection date (dd/mm/yyy)__/__/__	Others (Specify)……
V. Investigation requested:	
VI. History (including drugs used) **VII. Diagnosis**…… **Requesting clinician's Name**……Sign……Date(dd/mm/yyy)__/__/__	
Report (including macroscopic examination): **Test done by** (Name)……Sign……..Designation…….Date(dd/mm/yyy)__/__/__ **Approved by** (Name)……Sign……..Designation…….Date(dd/mm/yyy)__/__/__	

Appendix 3: Ethical clearance approval letters

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/180

8th December, 2016

Manyu Grace Chepkemoi
Reg. No. TM300- 1179/2012
J.K.U.A.T

Dear Grace,

Re: Approval of Annual Renewal – Comparison of Pap, Modified Pap and Gram Stained Cervico-vaginal Smears in the Diagnosis of Bacterial Vaginosis in Women Attending Thika District Hospital (P613/12/2013)

Refer to your communication dated 5th December, 2016.

This is to acknowledge receipt of your request and hereby grant you annual extension approval for ethics research protocol **P613/12/2013.**

The approval dates are 4th April 2016- 3rd April 2017.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH- UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH- UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

Protect to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chairperson, KNH-UoN ERC

COUNTY GOVERNMENT OF KIAMBU
DEPARTMENT OF HEALTH

Tel.Thika 067 21621/2 fax 21778
All correspondence should be addressed to
MED.SUPT.
When replying please quote

Ref: NO. MOMS/TKA VOL III (344)

THIKA LEVEL 5 HOSPITAL
P.O. BOX 227
THIKA

Date: 7th November, 2016

APPROVAL TO CARRY OF RESEARCH

Principle investigator: **MANYU GRACE CHEPKEMOI**

Research topic: **COMPARISON OF PAP, MODIFIED PAP AND GRAM STAINED CERVICO-VAGINAL SMEARS IN THE DIAGNOSIS OF BACTERIAL VAGINOSIS IN WOMEN ATTENDING THIKA LEVEL 5 HOSPITAL**

Following deliberations by Thika Level 5 hospital research committee, your proposal to carry out the above research at this facility has been approved. However, you will need to provide us with licence from NACOSTI before you can commence the data collection.

Take note that you are required to submit a copy of your research findings upon completion of the study to the hospital. It is also expected that the Ethical consideration and the research subjects confidentiality will be maintained as you have outlined in your proposal.

Any patient confidential information that you may access during your research should not be used without consent.

This letter is valid up to 7th November, 2017.

For any queries feel free to contact the committee chair though the Medical Superintendent's office. Thank you and all the best.

DR. J. WANGECHI
CHAIR TREC
THIKA LEVEL 5 HOSPITAL N

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
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9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No **NACOSTI/P/16/72508/12891**

Date: **2nd August, 2016**

Grace Chepkemoi Manyu
Jomo Kenyatta University of Agriculture
And Technology
P.O. Box 62000-00200
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Comparison of pap, modified pap and gram stained cervico-vaginal smears in the diagnosis of bacterial vaginosis in women attending Thika District Hospital,"*** I am pleased to inform you that you have been authorized to undertake research in **Kiambu County** for the period ending **2nd August, 2017.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Coordinator of Health, Kiambu County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

BONIFACE WANYAMA
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

The County Coordinator of Health
Kiambu County

Appendix 4: Research publication

International Journal of Scientific and Research Publications, Volume 8, Issue 12, December 2018 618
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Comparison of PAP, Modified Pap and Gram Stained Cervico-Vaginal Smears in the Diagnosis of Bacterial Vaginosis in Women Attending Thika District Hospital

G.C. MANYU[1], L.W. MUCHIRI[2] and M.N. KAHATO[3]

DOI: 10.29322/IJSRP.8.12.2018.p8479
http://dx.doi.org/10.29322/IJSRP.8.12.2018.p8479

ABSTRACT

Objective: To compare Pap, Modified Pap and Gram stained cervico-vaginal smears in the diagnosis of bacterial vaginosis in women to establish if Modified Pap was a suitable alternative to Pap method.

Design: Descriptive cross-sectional survey of bacterial vaginosis with a comparative evaluation of three methods.

Setting: Thika District Level Five (County) Hospital.

Subjects: A total of 150 female patients who consulted for services at Antenatal Care and Family Planning clinics at Thika district Level 5 (County) hospital between November 2016 and May 2017and who met the inclusion criteria were recruited into the study.

Main outcome measures: Presence or absence of bacterial vaginosis.

Results: The study showed that Pap and Modified Pap methods yielded sensitivity of 47.6% and 26.2%, positive predictive value (PPV) of 80.0% and 68.8%, negative predictive value (NPV) of 82.4% and 76.9%, likelihood ratio of positive result (LR+) of 10.3 and 5.69, likelihood ratio of negative result (LR-) of 0.55 and 0.77 respectively and specificity of 95.4% and overall diagnostic accuracy of 38.9% for both methods.

Conclusion: The Modified Pap staining method has diagnostic value when it is positive in diagnosis of bacterial vaginosis and can therefore be a suitable alternative to Pap method as a confirmatory test for bacterial vaginosis.

INTRODUCTION

Bacterial vaginosis (BV) is a vaginal infection caused by imbalance in the normal vaginal flora. It is characterized by low levels of normally predominant *Lactobacilli species (spp),*which is replaced by *Gardnerella vaginalis, Prevotella spp, Porphyromona spp, Bacteroides spp, Mobiluncus spp* and *genital Mycoplasma spp*.[1]. It is among the most common reproductive tract infections in women worldwide [2]. Estimated prevalence of BV ranges from 20% to 50% in African populations [3], with higher levels being documented in female sex workers [4].

Until recently, BV which was originally thought to be of little long-term clinical significance, has been implicated in increasing the risks of preterm birth [5], development of pelvic inflammatory disease [6], pregnancy loss, still births, gestational bleeding, preterm birth, preterm labour, premature rupture of membranes, amniotic fluid infection, postpartum endometritis and post caesarean wound infections [7].

Recent studies have shown that Pap stained cervico-vaginal smears can be used to diagnose BV [8-10] and can be a wholly adequate alternative to Gram-stained smears [11] hence the need to validate its use for diagnosis of BV. Therefore, this study sought to establish if Modified Pap method can be used to diagnose BV in cervico-vaginal smears and if Modified Pap method is a suitable alternative to Pap method in diagnosis of BV in cervico-vaginal smears.

MATERIALS AND METHODS

This descriptive cross-sectional study was done at Thika District Level 5 (County) Hospital's family planning (FP) clinic, ante-natal clinic (ANC) and medical laboratory. Thika District Level 5 (County) Hospital is a government hospital located in Thika town, Kenya and serves as a referral hospital for neighbouring districts and also as a teaching hospital.

The inclusion criteria were all females aged 18-45 years (child bearing age), sexually active, had no vaginal bleeding at the time of study and who gave voluntary consent to participate. The exclusion criteria were all females who at the time of the study had not met the inclusion criteria. Ethical clearance was given by Kenyatta National Hospital/University of Nairobi (KNH/UON) Ethics and Research Committee and also by Thika District Hospital, where the study was conducted, as well as by the National Commission for Science, Technology and Innovation (NACOSTI).

A sample size of 150 women was determined statistically and women who met the inclusion criteria were informed about the study and consent obtained through signing informed consent form. A structured questionnaire was then administered to all the subjects to obtain and record socio-demographic information (age, occupation, residence, education, marital status and number of sexual partners), reproductive history, vaginal and menstrual hygiene practices as well as clinical history.

Cervico-vaginal smear was then collected from the posterior fornix and lateral vaginal wall from each participant using a cervical scraper, but in women who were pregnant or suspected to be pregnant, sampling was restricted to lateral vaginal wall. The colour of the discharge (if present) was first noted and documented.

Three smears were prepared from each cervical scraper; two of the smears were fixed in 95% alcohol for 15 minutes and then stained with Pap and Modified Pap methods respectively. Pap staining method protocol consisted of 15 dishes and Modified

Pap staining method protocol consisted of 14 dishes with the smears held in respective dishes for 10 seconds (dips) with blotting done in between changes from one dish to the next. Mounting was done using DPX mountant and coverslip attached with overnight drying (Table 1). The air dried smear was stained using Gram stain method (Table 2).

Blinding of the staining method used was done using unique codes to label the smears. The three sets of smears were examined using light microscopy with the threshold for a positive BV diagnosis being presence of >20% clue cells in Pap and Modified Pap stained smears and a 7-10 Nugent score of bacterial morphotypes in Gram stained smears.

Primary examination of the smears was done by the principal investigator (PI) using Bethseda System 2001 of reporting cervico-vaginal smears for Pap stained smears and Nugent classification system for bacterial morphotypes in the Gram stained smears. In order to minimize intra- and interobserver variability, two cytologists and two microbiologists confirmed the Pap stained smears and Gram stained smears respectively. It is only at the end of this that the microscopists revealed their reports and any discrepancies resolved.

RESULTS

All the three sets of smears prepared from the sample of 150 were found to be satisfactory for evaluation and data analysis was done using 95% confidence interval (CI) and a statistically significant P-value of less than 0.05.

The mean age of the subjects was 26.9 years with a median of 25 and standard deviation (SD) of 5.9. The minimum and maximum ages were 19 and 42 years respectively. Majority of the subjects, 55.3% were 18-25 years old, followed by 31.3% who were 26-35 years while the least number, 13.3% were 36-45 years old (Figure 1).

Gram stain method using Nugent's scoring system which was the diagnostic gold standard in this study was able to detect forty two (28%) positive cases of BV. There were 6 subjects with intermediate flora and were counted as negative for BV. On the other hand, out of 150 smears stained with both Pap and Modified Pap methods, twenty five (16.7%) and sixteen (10.7%) smears respectively were BV positive with presence of >20% clue cells which was the threshold for positive BV diagnosis. Majority of BV positive cases using Gram stain method were in the age group of 26-35 years with twenty (47.6%) cases. The results indicate that there is a difference in diagnosis of BV in cervico-vaginal smears between the three staining methods, Pap, Modified Pap and Gram stain (Figure 2).

The results of Pap and Modified Pap methods were compared to the results of Gram stain method using Nugent's scoring system which was the confirmatory diagnostic gold standard test in this study and which was administered concurrently with the other two methods to each participant. A positive result in Pap and Modified Pap methods was considered "true positive" if it was confirmed positive using Gram stain method; a negative result in Pap and Modified Pap methods was considered "true negative" if it was confirmed negative using Gram stain method; a positive result in Pap and Modified Pap methods was considered "false positive" if it was confirmed negative using Gram stain method and a negative result in Pap and Modified Pap methods was considered "false negative" if it was confirmed positive using Gram stain method.

Pap method showed BV in twenty (20/42) subjects who tested positive on Gram stain method giving a sensitivity of 47.6%. On the other hand, Pap method showed negative results for BV in twenty two (22/108) subjects with negative results on the Gram stain method giving a specificity of 95.4%. Twenty two subjects who tested negative on Pap method and had BV on Gram stain method gave a false negative rate (FNR) of 52.4% while five subjects who had BV on Pap method and a negative result on Gram stain method gave a false positive rate (FPR) of 4.6% . Positive predictive value (PPV) was 80.0% while the negative predictive value (NPV) was 82.4%. The likelihood ratio for a positive test (LR+) was 10.3 while likelihood ratio for a negative test was 0.55. The overall diagnostic accuracy was 38.9% (Table 3). Modified Pap method showed BV in eleven (11/42) subjects who tested positive on Gram stain method giving a sensitivity of 26.2%. On the other hand, Modified Pap method showed negative results for BV in thirty one (31/108) subjects with negative results on the Gram stain method giving a specificity of 95.4%. Thirty one subjects who tested negative on Modified Pap method and had BV on Gram stain method gave a false negative rate (FNR) of 73.8% while five subjects who had BV on Modified Pap method and a negative result on Gram stain method gave a false positive rate (FPR) of 4.6% . Positive predictive value (PPV) was 68.8% while the negative predictive value (NPV) was 76.9%. The likelihood ratio for a positive test (LR+) was 5.696 while likelihood ratio for a negative test was 0.77. The overall diagnostic accuracy was 38.9% (Table 4).

The results show that Pap and Modified Pap methods vary in sensitivity, 47.6% and 26.2% respectively, but had similar specificity of 95.4%. However, even though their NPV's were fairly close, PPV of Pap method, 80.0% was relatively higher than 68.8% of Modified Pap. This indicates that Modified Pap method can be a suitable alternative to Pap method especially in excluding BV in truly negative cases. The diagnostic capabilities of Pap and Modified Pap methods in the diagnosis of BV in cervico-vaginal smears were analyzed using Cohen kappa statistics to determine the consistency between the two methods to establish if Modified Pap was a suitable alternative to Pap method in this case. The results indicate that there is a statistically significant ($p \leq 0.05$) level of agreement between the two methods with the kappa value of 0.692 representing moderate agreement between Pap and Modified Pap methods in diagnosis of BV indicating that Modified Pap method can be a suitable alternative to Pap method in diagnosis of BV (Table 5).

Table 1: Pap and Modified Pap staining protocols

Pap protocol		Modified Pap protocol	
Tap water	10 dips	1% acetic acid	10 dips
Harris Haematoxylin	10 dips	Pre-heated Harris's	10 dips
Tap water	10 dips	Haematoxylin (60^0C)	10 dips
95% ethanol	10 dips	Tap water	10 dips
OG-6 stain	10 dips	1% acetic acid	10 dips
95% ethanol	10 dips	OG-6	10 dips
EA-50	10 dips	1% acetic acid	10 dips
95% ethanol	10 dips	EA-50	10 dips
95% ethanol	10 dips	1% acetic acid	10 dips
100% ethanol	10 dips	Methanol	10 dips
100% ethanol	10 dips	Methanol	10 dips
100% ethanol	10 dips	Methanol	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	Xylene	10 dips
Xylene	10 dips	Xylene	10 dips
DPX mount and coverslip		DPX mount and coverslip	

Table 2: Gram stain method protocol

Heat fix air dried smear	
Crystal violet stain	1 minute
Tap water	
Gram's iodine	1 minute
Tap water	
Acetone-alcohol	6 seconds
Tap water	
Neutral red	2 minutes
Tap water	
Air dry	

http://dx.doi.org/10.29322/IJSRP.8.12.2018.p8479

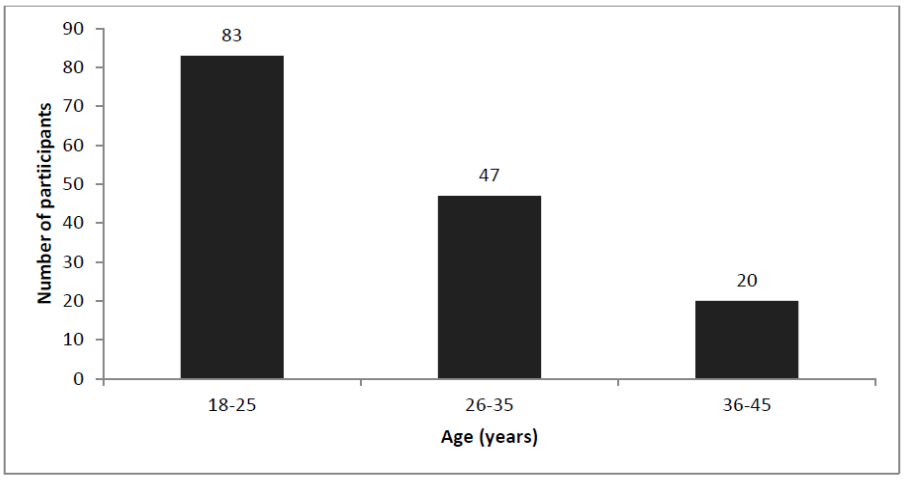

Figure 1: Age distribution of the subjects

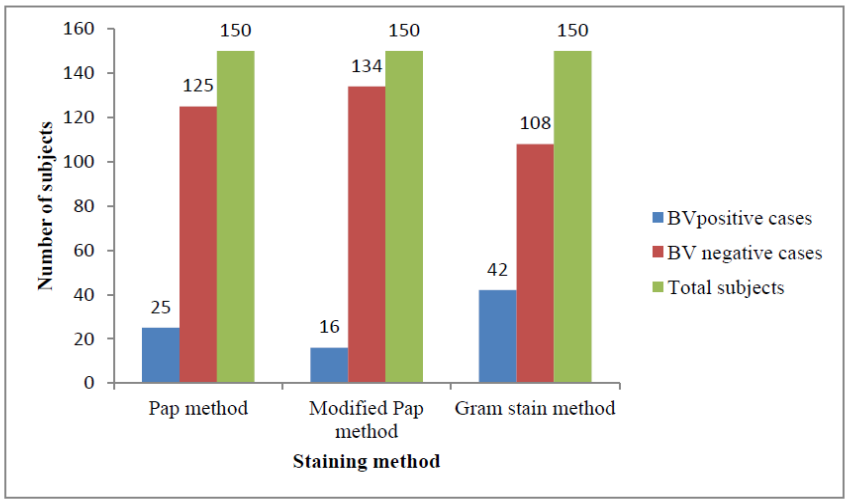

Figure 2: Frequency distribution of BV positive and negative cases in cervico-vaginal smears stained using Pap, Modified pap and Gram stain methods

Table 3: Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Pap method with reference to Gram stain method as the gold standard in the diagnosis of BV

			Gram stain method		
			Positive	Negative	Total
Pap method	Positive	Count	20	5	25
		% within Pap_method	80.0%	20.0%	100.0%
		% within Gram stain method	47.6%	4.6%	16.7%
	Negative	Count	22	103	125
		% within Pap_method	17.6%	82.4%	100.0%
		% within Gram stain method	52.4%	95.4%	83.3%
Total		Count	42	108	150
		% within Pap_method	28.0%	72.0%	100.0%
		% within Gram stain method	100.0%	100.0%	100.0%

*Pap_method * Gram stain method crosstabulation.

Table 4: Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of Modified Pap method with reference to Gram stain method as the gold standard in the diagnosis of BV

			Gram stain method		
			Positive	Negative	Total
Modified Pap method	Positive	Count	11	5	16
		% within Modified Pap_method	68.8%	31.2%	100.0%
		% within Gram stain method	26.2%	4.6%	10.7%
	Negative	Count	31	103	134
		% within Modified Pap_method	23.1%	76.9%	100.0%
		% within Gram stain method	73.8%	95.4%	89.3%
Total		Count	42	108	150
		% within Modified Pap_method	28.0%	72.0%	100.0%
		% within Gram stain method	100.0%	100.0%	100.0%

*Modified Pap method * Gram stain method crosstabulation.

Table 5: Cohen kappa measure of agreement for Pap and Modified Pap methods

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	.692	.086	8.753	.000
N of Valid Cases		150			

*Modified Pap method * Pap_method Crosstabulation Symmetric Measures
a. Not assuming the null hypothesis.
b. Using the asymptotic standard error assuming the null hypothesis.

DISCUSSION

Pap smear test is a simple cytology screening test primarily used in detection of pre-neoplastic and neoplastic changes in the uterine cervix. In reporting cervical pap smear results, a remark is usually made on presence of cervico-vaginal infection due to bacteria, fungi and candida with the Bethesda system having a class on reporting 'shift in vaginal flora, suggestive of BV' [12]. Conventional Pap method has undergone several modifications to reduce alcohol use to make it cost effective in resource poor settings. One of the modified Pap protocols is Rapid, Economic, Acetic acid, Papanicolaou (REAP) method [13] that has successfully been utilized in screening for cervical cancer in Pap smears with no compromise on staining quality and diagnostic standards.

This study was to compare Pap, Modified Pap and Gram stained cervico-vaginal smears in the diagnosis of BV to establish if Modified Pap was a suitable alternative to Pap method in this regard.

The subjects in the study were 150 (n=150) from whom three sets of smears were prepared and found satisfactory for evaluation. The age range of the female subjects was 19-42years, with majority in the 18-25 years old age group. Mean age of the subjects was 26.9 years with a median of 25 and standard deviation (SD) of 5.9. This is comparable to a study done by Shayo *et al.,* [14] in Mwanza, Tanzania where median age of the subjects was 26 years. In the present study, BV was detected in 28% of the women and this is similar to a study in Ghana by Aubyn *et al.,* [15] that reported BV prevalence of 28%. This present study utilized Gram stain method as the reference diagnostic standard in evaluating diagnostic performance of Pap and Modified Pap methods and reports sensitivity of 47.6% and 26.2%, specificity of 95.4% and 95.4%, PPV of 80% and 68.8% and NPV of 82.4% and 76.9% for Pap and Modified Pap methods respectively. The results for Pap method are comparable to those reported by Platz-Christensen *et al.,* [16] of sensitivity, specificity, PPV and NPV values of 88.2%, 98.6%, 96.8%, 94.7% respectively and concurs with Livengood [17] who reported that Pap test has sensitivity as low as 50% and specificity of about 95% in diagnosis of BV indicating that a positive result is reliable evidence of BV presence but a negative result does not exclude presence of BV. However, these results differ from that of a prospective study done by Karani *et al.,* [18] in Mombasa, Kenya that reported sensitivity, specificity, PPV and NPV values of 59.4%, 83.3%, 67.3% and 78.0% respectively. The varied results may be attributed to interobserver variability, type of population used, environment and other socio-demographic characteristics of the subjects, research design and specimen source site (cervix/endocervix as opposed to posterior fornix and lateral vaginal wall).

In this study, a kappa value of 0.692 showed moderate agreement between Pap and Modified Pap methods with the overall diagnostic accuracy of 38.9% for both methods. Additionally, Filho *et al.,* [19] reported that Pap method would be a valid diagnostic option in comparison to gold standard when it especially gives a positive BV result and a mean specificity of 95% and this criterion has also been fulfilled by Modified Pap method.

CONCLUSION

In conclusion, this study faced several limitations that will affect the generalizability of the results. First, due to limited resources and time in conducting this research, the research subjects were recruited from only one hospital during the study period and this may not be representative of the annual female population served by the hospital. Secondly, only women visiting ANC and FP clinics were recruited and the results may not be applicable to women delivering at the hospital but this will be augmented when the research can be applied to other populations of women. Thirdly, due to recall or social desirability bias, self-reported information may have been misreported or under-reported during the questionnaire interview and lastly, extensive training requirements made it impossible to perform Nugent scoring of Modified Pap and Pap methods .

In spite of these limitations, the greatest strength of this study is that it showed that Modified Pap has diagnostic value for BV diagnosis when it is positive and is therefore suitable as a confirmatory test for BV. Therefore, this study supports the use of Modified Pap as an alternative to Pap method in diagnosis of BV in cervico-vaginal smears.

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